QUINONE METHIDE PRECURSORS AS ACETYLCHOLINESTERASE REALKYLATORS:
SYNTHESIS AND KINETIC STUDIES OF QUINOLINE FRAMEWORKS

RESEARCH THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Bachelor of Arts with *research distinction* in Chemistry

In the College of Arts and Sciences at The Ohio State University

By

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2018

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ABSTRACT

Organophosphorus (OP) agents are responsible for the inhibition of the enzyme acetylcholinesterase (AChE). AChE is responsible for the hydrolysis of the neurotransmitter acetylcholine. OPs are covalent inhibitors of AChE, and OP compounds have been used as chemical warfare agents as well as pesticides. Without functioning AChE, acetylcholine will accumulate and can lead to serious adverse health effects, such as vomiting, paralysis, and even eventual death by respiratory failure. The magnitude of the effects and rate of death are determined by the OP's toxicity. There are known therapeutics, called pyridinium oximes, which can reverse the inhibition and reactivate AChE if administration occurs prior to the aging time frame. If left untreated, aging will occur, which is a dealkylation of the phosphylated serine residue in the active site to form an anionic phosphonate (or phosphate) serine. Aging forms a stable, anionic phosphylated intermediate and that species has been recalcitrant to reactivation by oximes. Thus, there are currently no effective treatments to reverse the aging process.

Our research focuses on synthesizing and testing small organic compounds that can hopefully re-alkylate the OP-aged enzyme and enable it to be reactivated by pyridinium oximes. Quinone methide precursors (QMPs) are of particular interest because their structures resemble that of other molecules that are capable of binding to the active site of AChE. Furthermore, QMPs have been shown to have the ability to alkylate structures like phosphodiesters, DNA and proteins, so they could potentially realkylate aged AChE as well. Their reactivity can be intensified by the addition of other

functional groups to their framework. Many libraries of QMPs have been synthesized, specifically libraries derived from different quinoline and isoquinoline frameworks. The aim is that one of these synthesized derivatives will be the key to realkylating aged AChE efficiently, and eventually lead to the reactivation of the enzyme. I will present multiple synthetic efforts towards the assembly of QMPs, as well as their screening assays with OP-aged AChE.

ACKNOWLEDGEMENTS

First and foremost, I must thank my advisor, Dr. Christopher Callam, for offering me the opportunity to work in his laboratory; this thesis would surely not exist without his unwavering support and constant advice. He inspired a love for organic chemistry and synthesis in myself since our first meeting. The scientific independence he offered me allowed me to cross the boundaries of what I ever believed possible of myself, and took my scientific creativity and inquiry to levels beyond the average undergraduate researcher, and inspired me to treat this project as if I was a PhD candidate. I thank him for his mentorship and friendship these past few years and for helping me more than I could ever repay, or he could ever realize. I hope in the years to come to be a fraction of the person he is; his endless selflessness, superior intelligence, and relenting kindness remind me of the good in this world, and remind me of the type of scientist and human being I hope to be in the future. His endless sacrifices solely to benefit me, my project, and my future will never be forgotten.

I am indebted to Dr. Christopher Hadad and would like to take this time to thank him as well. He always had an open door and was always quick to help offer advice for any and all of my endeavors. I surely would be on the wrong path to medical school without his guidance. I thank him for treating me like the graduate students in his lab, always listening to my opinion, allowing me the independence to complete this project, and to even be on some of his research papers. I am eternally grateful for his support and smiles these past years, and can never repay what he has done for me.

I jointly thank Dr. Hadad and Dr. Callam for enabling my research to take me around the country and the globe. With their guidance, I was able to travel to four states outside of Ohio to present my research, as well as to Germany and fifteen other countries in Europe. I have so many fond memories from these travels, and will surely never be able to repay them for these opportunities. I also thank them for allowing me to present my research often in Ohio and pursue and fulfill my dreams of chemistry and research before going off to medical school.

I would like to thank the other members of the research group for their help these past years and for making the hours in lab fly by. I thank Bill Coldren for his helpful calculations, and even more helpful sense of humor. I thank Qinggeng (Albert) Zhuang for his help with all of the kinetic studies and for always being a friendly face, and notably for fishing out needles from reagents when I dropped them often when I first started. I thank Andrew Franjesevic for his endless advice and friendship during my long days in lab, and for always taking time out of his busy days to teach me a new technique or talk about an interesting paper with me. I thank Tom Corrigan for his help while he was here in guiding my early syntheses, and always looking up reagents for me when I could not find them.

I would also like to thank the other undergrads who have been my companions in lab during my time, notably Nathan Yoshino, Justin Smith, Jenna Tabbaa, Dennis Yang, Ravali Kode, Maura Kopchak, Rachel Dicken, and Ashley DeYong. I thank Rachel Dicken for informing me about the lab in the first place; I certainly would not be here without her. I thank Justin for his training when I first started, and for Dennis for allowing me to train him. I thank Jenna for always calming me down during this thesis, and being

my companion for some of my travels and presentations. I thank Nathan for being a great friend my first summer in lab.

I would like to especially thank Milauni Mehta for sharing my love for chemistry and research. Her companionship these past few years, whether in Germany, at various conferences around the US, poster presentations here at OSU, or just in the hallways of CBEC made my experience unique and very special. I would never have survived this thesis, or this research project in general, without her, and am humbled and honored to call her my friend.

I would like to thank the people who have continuously encouraged my adoration of chemistry and have taught me the knowledge that allowed me to complete this thesis. Most notably, I thank Dr. Noel Paul for his constant support throughout my research. He was always willing to offer insight and advice, and the knowledge I gained from him is what allowed some of these syntheses to be possible. I would like to thank Dr. Christo Sevov, Dr. David Nagib, Dr. Thomas Magliery, Dr. Terry Gustafson, and Dr. Rosemary Loza for their parts in inspiring my love for chemistry and being such great role models to me over these past few years, I surely would not be here without you all.

I would like to thank The Ohio State University Department of Chemistry for their financial support and generous donations that allowed not only the completion of this project, but for vast opportunities to share my research in Ohio and abroad.

Lastly, I would like to thank my friends and family, who over these years have done nothing but support me and listen to me talk about this project without even understanding any of the words I was saying. Without you none of this would have been possible.

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PUBLICATIONS

Zhuang, Q.; Franjesevic, A.; Corrigan, T.; Coldren, W.; Dicken, R.; Sillart, S.; DeYong, A.; Yoshino, N.; Smith, J.; Fabry, S.; Fitzpatrick, K.; Blanton, T. G.; Joseph, J.; Yoder, R. J.; McElroy, C. A.; Ekici, Ö., D.; Callam, C; Hadad, C. "Demonstration of in vitro Resurrection of Aged Acetylcholinesterase after Exposure to Organophosphorus Chemical Nerve Agents". *J. Med. Chem.* **2018**, *manuscript in review*.

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FIELDS OF STUDY

Major Field: Chemistry

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CHAPTER ONE

INTRODUCTION

1.1 The Fruition of Organophosphorus Nerve Agents: A Historical Background

Following the First World War, Germany found itself in a state of complete distress and devastation. The Third Reich strategists desired to become more self-independent during this time of desolation and strove to reduce Germany's reliance on imported food. Gerhard Schrader, a chemist working at the I.G. Farben chemical company, had been assigned the job of synthesizing new insecticides in order to prevent insects, specifically wooly aphids, from continuing to harm the food produced.[1] At first, the pesticides were attempted to be based from fluorine and sulfur, but as both failed, Schrader switched to phosphorus- and cyanide-based derivatives.

On December 23, 1936, Schrader synthesized "Preparation 9/91", which proved to be highly toxic and severely deadly[2]. Schrader himself after working with small quantities of sample was hospitalized for a variety of symptoms, among those being difficulty breathing, vision trouble, and dizziness, and his coworkers who also became exposed were overcome with similar symptoms – all involved took weeks to recover. The compound he made, ethyl-dimethyl-amido-phosphoro-cyanidate, known today as tabun (dubbed as such from the German word for taboo), seemed to affect the nervous system in a way that the victim's bodily functions were no longer under the brains control. An early sample was administered as a vapor to apes with a fatal effect, and was therefore deemed lethal to warm-blooded animals and consequently a poor insecticide, as it did not selectively kill pests. However, due to its obvious toxicity to humans, I.G. Farben alerted the German military about the potential of this compound

as a weapon instead of a pesticide. Schrader discovered a new class of toxic chemicals known as nerve agents, and tabun (deemed GA), became the first in the G-series, “G” standing for “German”.

Despite the peace accord via Treaty of Versailles, German scientists secretly developed weapons of mass destruction against orders. During the time of WWI, the chemical warfare agents utilized, like phosgene and mustard gas, took hours or even days to kill victims. The government did not miss the potential of this new agent, which could kill a human within twenty minutes. Scientists went to work studying the physiological effects of tabun and how to increase its lethality. In June 1939, Schrader appeared again at the forefront of this project with the development of Substance 146, or isopropyl-methyl-phosphono-fluoridate. The compound was later named sarin as an acronym for its four creators: *S*chrader, *A*mbos, *R*üdiger, and Van der L*in*de.[3] Although more complicated to synthesize, sarin (deemed GB) was found to be 500 times more lethal than cyanide. In order to deploy GA and GB in lethal doses, the Nazis developed bombs to be used to disperse the clear, odorless liquids as a mist to ensure enough is inhaled to be lethal. It is important to note that while referred to as nerve gases, nerve agents are actually liquids.

In 1943, Richard Kuhn was hired to research the mechanism of action of these new compounds. It was discovered they acted to block acetylcholinesterase, which results in the buildup of acetylcholine in synapses and the subsequent prevention of electrical termination of signals to muscles in the body due to the muscle cells being overstimulated by excess neurotransmitter, which accounts for the symptoms seen in the poisoned scientists, like pinpoint pupils and asphyxiation. During this research,

Kuhn screened a wide variety of organophosphorus agents to test the various levels of inhibition of the enzyme. Upon replacing the isopropyl group with a pinacolyl group, he discovered that he manufactured a more potent nerve agent than tabun, one that was even twice as lethal as sarin (Figure 1.1). Compound 25075[4], or 3,3-dimethyl-butan-2-yl-methyl-phosphono-fluoridate (deemed GD), deactivated acetylcholinesterase within two minutes and readily penetrated the skin to add another method of poisoning. It was later renamed soman.

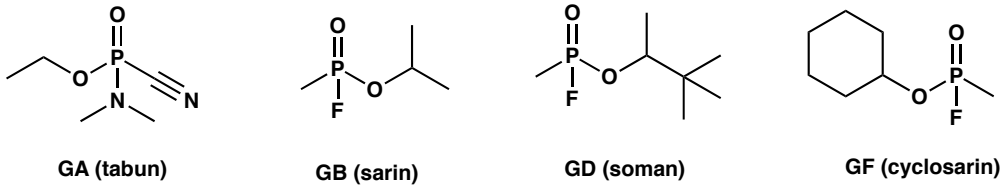


Figure 1.1: Structures of common G-series organophosphorus nerve agents

The Germans did not end up using these deadly agents during WWII, though they certainly had the means. Despite their chemical advantage, Hitler chose not to use the developed toxins to help them win the war. Some suspect it is due to his fear of retaliation by the allies with similar weapons, while others suspect that due to his past experiences with harmful gas while in military, he did not want to subject other soldiers to the same fate.[5] Shortly after the war ended, Russia found evidence of these chemical agents by uncovering old lab notebooks detailing the synthesis of sarin. During the 1950's during the Cold War, the United States and United Kingdom teamed up to discover more about these agents. They screened old nerve agents, like sarin and cyclohexyl-methyl-phosphono-fluoridate (deemed GF, or cyclosarin), and synthesized

new sarin-like derivatives to figure out what the perfect weapon would be. While the most expensive, soman was deemed to be the most lethal agent, but as sarin was still efficient and cheaper to synthesize, it was stockpiled instead.

After WWII, the field of insecticides and pesticides became a very popular field of research, especially for mites. In 1952, a researcher from the Imperial Chemical Industries, Dr. Ranajit Gosh, discovered a new class of nerve agents. Gosh was researching the use of organophosphorus agents as potential pesticides and he and J. F. Newman were making compounds containing nitrogen and sulfur. The compound was able to effectively kill lice and was placed on the market under the trade name Amiton.[6] Gosh suspected they might be deadly to humans so alerted the chemical warfare center in the UK. The UK denounced chemical warfare, but let the US in on the investigation of its lethality in exchange for information on nuclear devices. The company had to pull Amiton, or *O*,*O*-diethyl-*S*-(2-(diethylamino)ethyl)-phosphoro-thioate from the market for being too deadly. The compound became part of the V-Series, “V’ meaning “venomous”, and was renamed VG. A similar compound, ethyl-*N*-2-diisopropyl-aminoethyl-methyl-phosphono-thiolate, was developed in the partner company in the US, later renamed VX. Some other well known isomers include *S*-(diethylamino)ethyl-O-ethyl-ethyl-phosphono-thioate (deemed VE) and *S*-(2-(diethylamino)ethyl-*O*-ethyl-methyl-phosphono-thioate (deemed VM), though neither showed significant production in any country following their discovery (Figure 1.2). Studies showed the V-series agents to be far deadlier than the other gases that had been previously synthesized; in average temperature conditions they can persist for days, they have no smell, they can be a vapor or gas, and they only require 200

micrograms to kill a human being, making these agents the mostly deadly to mankind at this point.[7] VX proved durable for months in cold temperatures, so it was the US's weapon of choice and the nerve agent they stockpiled in case they needed it during the Cold War.

On March 14, 1968, six thousand sheep were found dead and seriously injured in Utah. It seemed that a nerve agent from the Army's testing site had drifted off the base into Skull Valley after they had been testing its ability to be dispersed as an aerosol via jets over a testing ground.[8] The sheep started acting strange, dazed, jerking, and often found on the ground unable to rise. The veterinarians on the case had never seen anything like this before, and soon discovered traces of organophosphates in the sheeps' bodies and in the field. All of the sheep were found to have depressed levels of acetylcholinesterase in their blood. This key symptom is common in pesticide and nerve agent poisoning, and was traced to VX after matching the sample with the traces of agent found in the sheep and in the field.

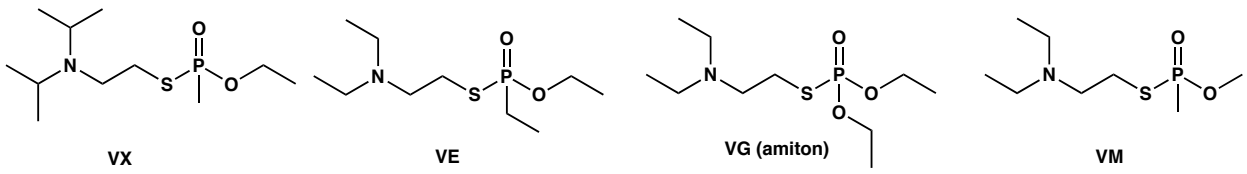


Figure 1.2: Structures of common V-series organophosphorus nerve agents

The Soviet Union desired to catch up to the allies in terms of chemical weapons. At the Scientific Research Institute No. 42, S. Z. Ivin, L. Soborovsky, and I. D. Shilakova developed Substance 33, an isomer of VX.[9] *N,N*-Diethyl-2-(methyl-(2-methylpropoxy)phosphoryl)-sulfanyl-ethanamine, renamed VR or "Russian VX", was

found to have a similar lethal dose to VX, while having a shorter window of treatment due to its rapid denaturation of acetylcholinesterase. At this point, all of the major world powers had their hands on stockpiles of these chemical weapons of mass destruction.

The US was not the only country to experiment with the harmful organophosphorus nerve agents. In a chemical warfare facility in the UK in 1953, scientists dripped sarin onto the arms of six people who were sealed inside a gas chamber[10]. Ronald Maddison fell ill and died within an hour. The Ministry of Defense unlawfully murdered this man and this became one of the longest cover-ups in cold war history.

After WWII, the US began to develop organophosphorus pesticides in large quantities. Diethyl-(dimethoxy-phosphino-thioyl)-thio-butanedioate, also known as malathion, was produced in 1950. In 1951, Schrader continued developing new insecticides including demeton. It was a mixture of the thiono- and thioloisomers of *O,O*-diethyl-2-ethylmercaptoethyl-phosphoro-thioate, thereby introducing a new class of insecticides having a thioether group.[11] Today, a wide range of organophosphorus compounds are still available as insecticides. In the continued quest for an acceptable pesticide, other compounds were synthesized, such as chlorpyrifos, parathion, and tebupirimphos due to their lower environmental persistence compared to previously synthesized pesticides (Figure 1.3). The popularity increased after the ban of organochlorine insecticides in the 1970s. These compounds are used in very, very low concentrations as to pose less harm to the user and to be safe to use around food. Though dilute, there is still high potential for these pesticides to cause harm to agricultural workers who handle and apply these chemicals to crops. The FDA has

lowered the limits of pesticides in foods sold to consumers in the US, but the world has not all followed this trend, which leads to the high numbers of 3 million global cases of pesticide poisoning per year, with an astonishing 220,000 deaths per year.[12] The majority of these cases are restricted to developing countries, and though most cases are due to poor working conditions, lack of training in handling, and inadequate regulation, there are numerous cases involving intentional self-harm with these agents as well.

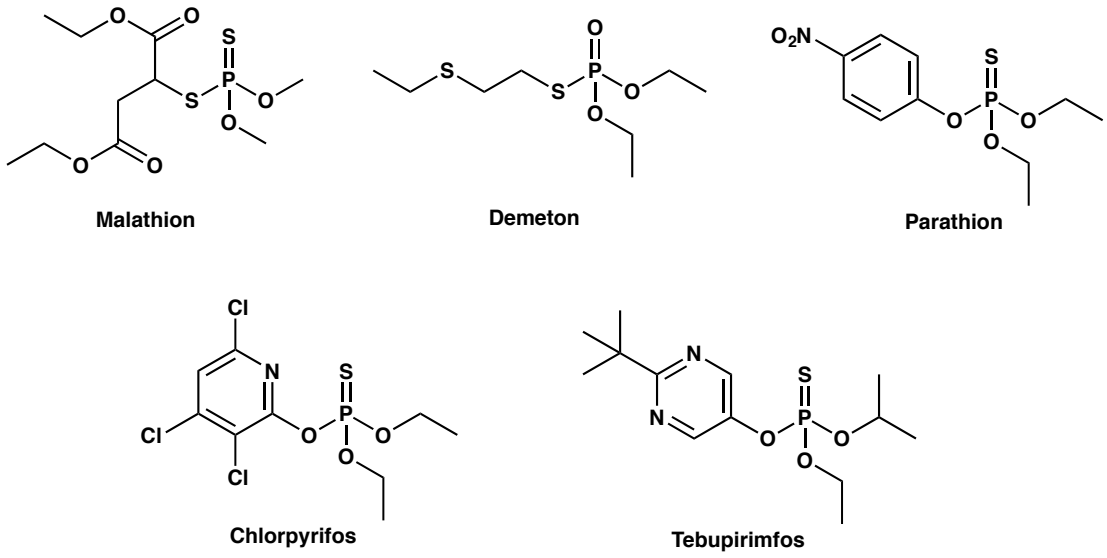


Figure 1.3: Examples of organophosphorus pesticides

During the Iran-Iraq War in the 1980s, Iraq used chemical warfare agents on Iran. They claim to have used 600 tons of sarin and 140 tons of tabun to take down enemy forces.[13] The onslaught killed nearly 5000 Iranians and over 100,000 were hospitalized. In 1988, Iraq even attacked its own citizens in Halabja, killing over 5000 and injuring over 7000.

On March 20, 1995, cult members from the Aum Shinrikyo sect punctured bags of homemade sarin on a subway in Tokyo, Japan[14]. They dropped five plastic bags of liquid sarin during peak rush-hour time in order to “speed up the pending apocalypse”. The subways looked like battlefields as injured patients fell out of the trains onto the ground, some even with blood gushing from their mouths. Though only a dozen people were killed, over 5,500 were hospitalized. If the sarin was deployed in a different way, it is hypothesized it could have killed thousands.

Syria has been associated the past few years with the use of organophosphates as war agents. Damascus, Syria was the location of a sarin attack on the morning of August 21, 2013, when rockets filled with the agent fell on the rebel suburbs of the Syrian capital, killing and injuring thousands.[15] The US and other countries had tried to prevent this by blocking sales of precursors of sarin. The UK had already licensed the sale of tons of sodium fluoride to Syria, which is suspect to have been used in the manufacture of these weapons. Around 1,429 were found dead, including 426 children. Syria was recently the site of another attack in April 4, 2017. More than 89 people were killed and another 541 injured in the rebel-held town of Khan Sheikhoun after the Syrian government’s air strike on that area.[16] When people started arriving to help, they inhaled the gas and instantaneously died. There was no smell, but people were found on the floor, unable to move with constricted pupils. In all of these victims, traces of one of the decomposition products of sarin, isopropyl-methyl-phosphonic acid (IMPA), were detected in the urine and blood of the victims.

On February 13, 2017, Kim Jong-nam was murdered in an airport in Kuala Lumpur. Kim is the estranged half-brother of Kim Jong-un, the North Korean leader.[17]

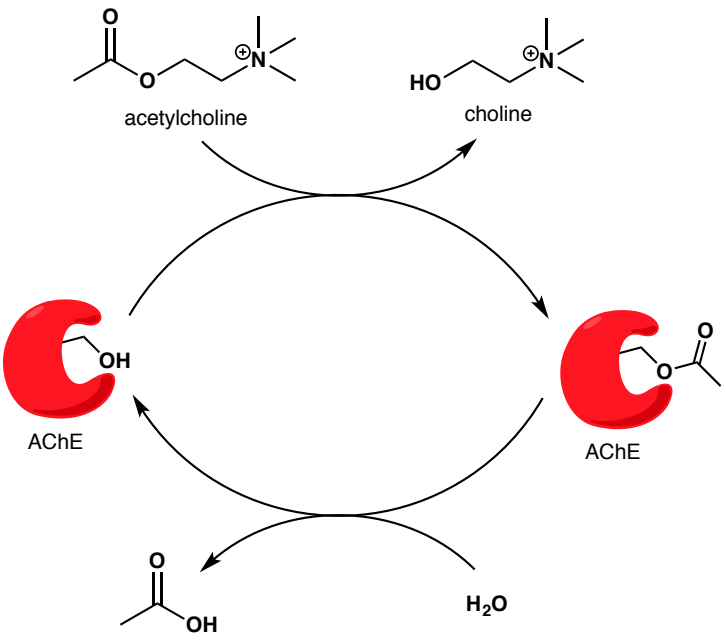
He was attacked by two women who smeared the substance VX on his face when he checked in for a flight. He died on the way to the hospital. The female attackers immediately washed their hands to prevent becoming ill themselves, though one was seen sick and vomiting shortly after the attack.

During the compilation of this thesis, a Russian spy Sergei Skripal and his daughter were found in the UK unconscious on a bench on March 4, 2018 and had no control of their bodily functions[18]. Both are still under critical condition and there is proof the murder weapon was a nerve agent. The identity of the specific nerve agent is thought to be VR, generated through a binary Novichok agent that contained compounds that easily combined on site to generate the nerve agent. Over twenty one doctors, first responders, and bystanders had to be treated as well. While all parties involved are alive and stable, Russia is suspected in the attempted murder of these individuals. It is clear that nerve agents are dangerous, lethal, and the threats to the citizens of the world are far from over. The need to find a cure for these poisons is large and should not be underestimated, as who knows the next time that these agents of mass destruction might be used next?

1.2 Acetylcholinesterase: Structure and Function

Acetylcholine (ACh) is a vital neurotransmitter utilized in the body. Acetylcholine is a small neurotransmitter that is released from a nerve cell and stimulates responses on other cells. When motor nerves get signals from the nervous system, they release acetylcholine into the synapses of muscle cells, which triggers the process of contraction. In order to terminate this signal to trigger muscle movement, the

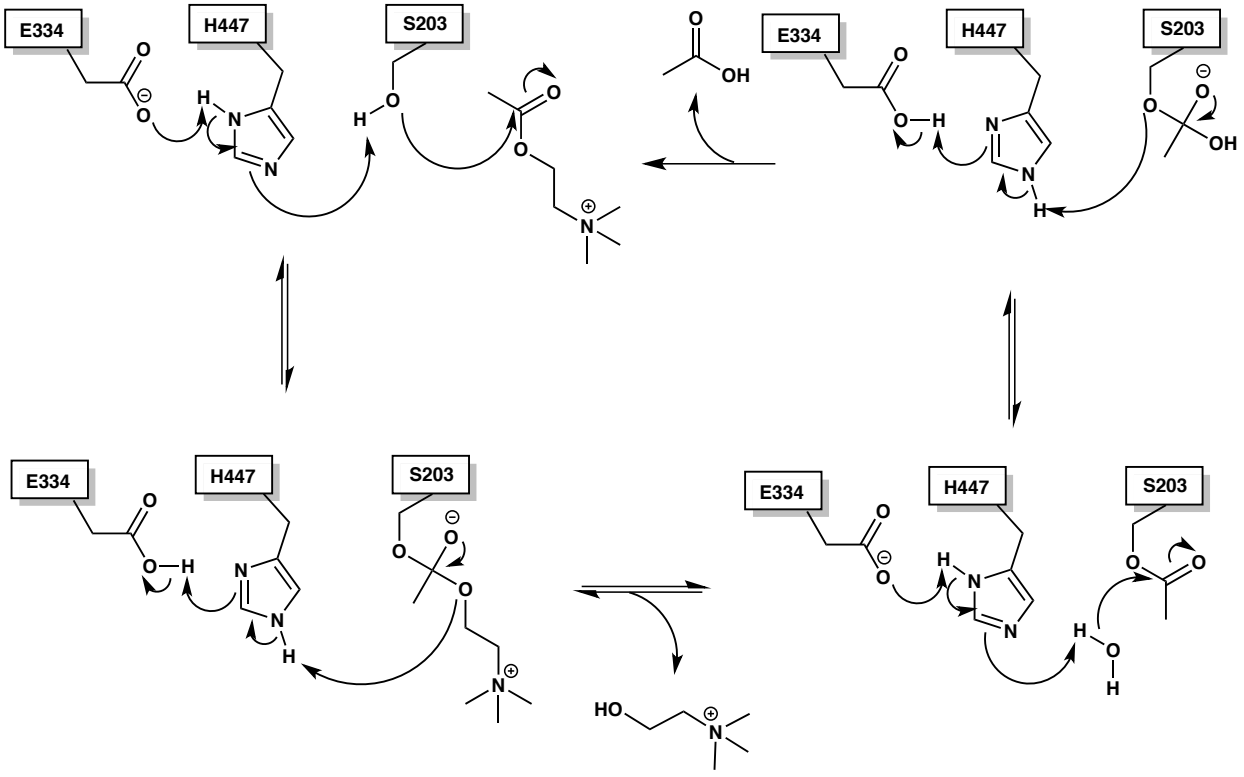
neurotransmitter must be removed or destroyed. The job of acetylcholinesterase (AChE), a serine hydrolase, is to hydrolyze acetylcholine and remove it from the synapse, and to maintain the desired levels of the ACh neurotransmitter in the synapse. ACh is found in the synapse of muscle and nerve cells, specifically in the synapses of the central nervous system, neuromuscular junctions in the peripheral nervous system, and bound to erythrocyte membranes in the blood. It catalytically breaks down acetylcholine into two component parts, acetic acid and choline (Scheme 1.1). This allows the signal to be stopped and these pieces to be recycled to build new neurotransmitter molecules. AChE has one of the fastest rates of any enzyme in the human body, hydrolyzing acetylcholine in about 80 microseconds.[19]



Scheme 1.1: Overall breakdown reaction of acetylcholine with the help of the Ser203 residue on AChE

The enzyme is responsible for the breakdown of 25,000 acetylcholine molecules per second. The catalytic efficiency (k_{cat}/K_M) of AChE is 1.50 x 10^9, which is a measure of the hydrolysis rate corrected for the binding affinity of the substrate.[20] This demonstrates a turnover rate that is one of the highest observed for any enzyme, approaching a value of a diffusion-controlled reaction rate. The enzyme contains a deep and narrow gorge that is about 20 Å long and extends halfway into the enzyme.[21] At the base, the opening widens and the active site lays 4 Å from the base. This is very surprising as due to the fast hydrolysis rate, it was assumed the active site must be on the surface. The gorge is too narrow to be able to transfer acetylcholine from the protein surface straight to the active site. What happens instead is a "breathing" motion that occurs in a 10 nanosecond period, and is a combination of side chain motions which allow the gorge radius to fluctuate in between 0.75 Å and 2.5 Å.[22] The active site has two parts, and "anionic" subsite and an "esteratic" subsite.[23] The anionic site interacts with the charged quaternary ammonium on acetylcholine via cation-pi interactions. The esteratic site contains the catalytic triad similar to other serine hydrolases, and is responsible for the turnover of acetylcholine. It contains a serine (203), histidine (447), and glutamate (334) residue, which form the catalytic triad. A large dipole has been identified along the gorge axis of 505 Debye.[24] This is hypothesized to drag acetylcholine from the anionic site, past the gorge, and into the catalytic site in the interior. This dipole can also help to explain how acetylcholine then becomes trapped in the gorge. It is further thought that a "backdoor" exit of the byproducts through a thin layer of the gorge wall is possible.[25] In the mechanism, the serine residue is activated through a hydrogen-bonding interaction between the residues, which allows the serine

residue to nucleophilically attack to the carbonyl of acetylcholine to form the vital tetrahedral intermediate (Scheme 1.2). This intermediate collapses to form choline and an acylated serine residue. The active enzyme is regenerated via reactions with water that restore the serine residue to its original state and release acetic acid.

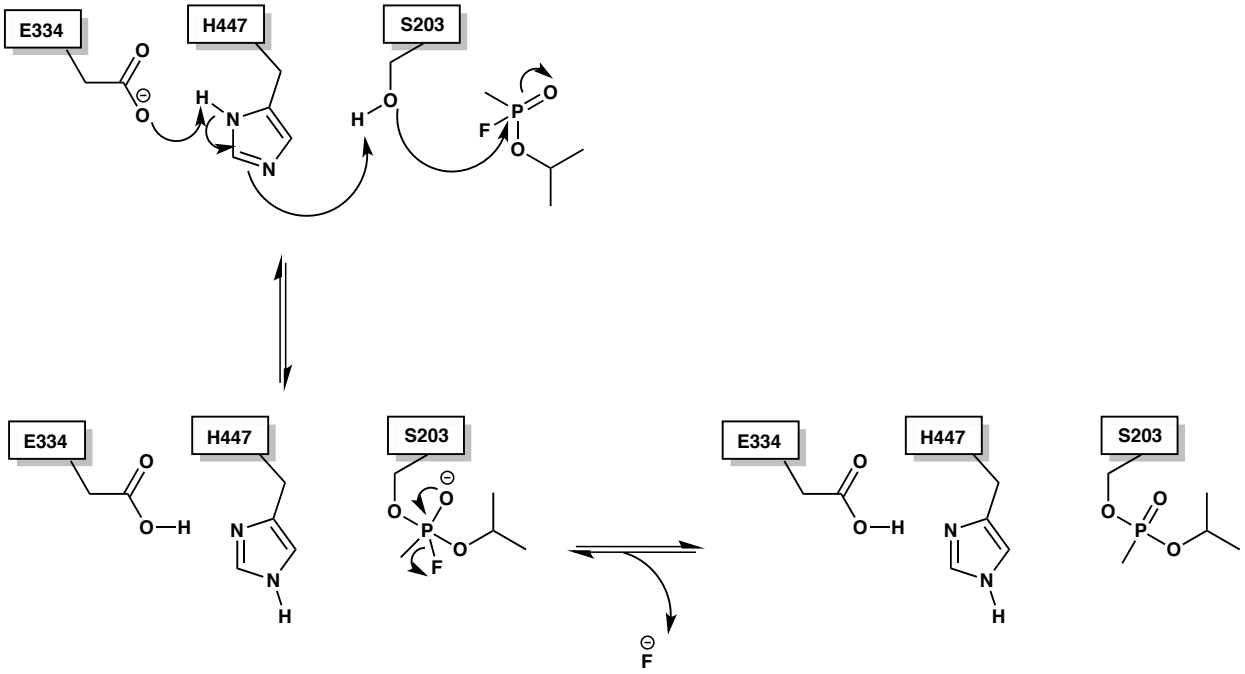


Scheme 1.2: The catalytic triad of acetylcholinesterase hydrolysis mechanism, begins at top left and circles counterclockwise

1.3 Acetylcholinesterase: Nerve Agent Inhibition

Organophosphorus nerve agents are substrate analogs to acetylcholine, and therefore they have the ability to irreversible inhibit AChE. The mechanism is similar to that of acetylcholine (Scheme 1.3). Upon elimination of the leaving group from the phosphorus group, a tetrahedral phosphonate (or phosphate) is formed in the catalytic

site on the serine residue. Due to nucleophilic attack being significantly slower on phosphorus than on carbon, the water normally used as the nucleophile to restore catalytic serine is not strong enough to cleave the phosphorus group from the catalytic site. It is also possible that the nearby H447 residue is forced into a conformation that does not promote the activation of the water molecule. The covalent modification of this serine residue results in the inability of AChE to further hydrolyze acetylcholine and is therefore now an inactive, inhibited (phosphylated) enzyme.



Scheme 1.3: Inhibition of the catalytic serine of AChE with sarin, an organophosphous nerve agent

The inactivation of AChE has some very negative side affects. As a result of this inactivation, acetylcholine builds up in the synapses, as there is nothing to reduce its concentration. As a result, the acetylcholine receptors of both muscarinic and nicotinic receptors are overstimulated. This results in initial excessive stimulation of both

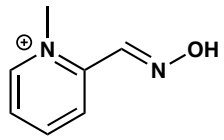
muscarinic and nicotinic receptors, followed by their depression. When the muscarinic receptors are overstimulated, the signs follow SLUDGE: salivation, lacrimation, urination, diaphoresis (sweating), gastrointestinal problems, and emesis (vomiting). Further progression leads to bronchospasm, bronchorrhea, blurred vision, fast or slow heart beat, hypotension, confusion and shock.[26] When the nicotinic receptors are overstimulated, skeletal muscle initially involuntarily contracts violently and irregularly. The inability to repolarize cell membranes results in weakness and paralysis, and severe reactions can lead to respiratory failure and eventual death, also known as "cholinergic crisis" (Table 1.1).

Exposure	AChE Inhibition	mAChR Symptoms	nAChR Symptoms	CNS Symptoms
Mild	0-60%	SLUDGE, nausea		Headache, dizziness
Moderate	60-80%		Fine skeletal muscle twitching	Psychosis, tremor, slurred speech, dysarthria
Severe	>80%		Muscle weakness, slowed reflexes, paralysis of diaphragm	Coma, convulsions, respiratory depression

Table 1.1: Signs and symptoms associated with organophosphorus exposure[27]

Strong nucleophiles, commonly in the form of pyridinium oximes, have been shown to reactivate the organophosphorus-inhibited enzyme (Figure 1.4). However, once the enzyme reaches a second inhibitory state, it was noted that there was a

significant decrease in their effectiveness after a certain period of time, and eventually the enzyme had zero ability to be resurrected. The state became known as “aged”.



2-PAM

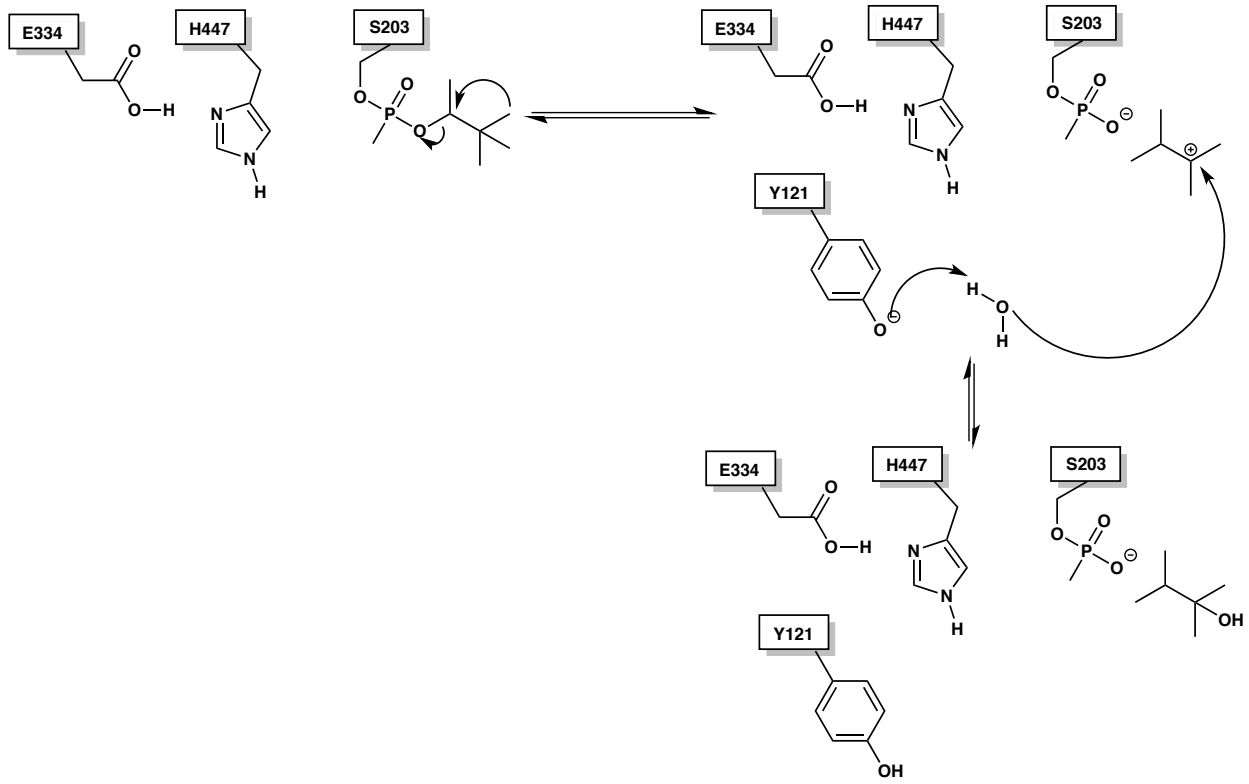
Figure 1.4: A commonly used pyridinium oxime, 2-PAM

Further studies were done on the aged state of AChE. It was discovered that the decrease of efficacy associated with this state was due to dealkylation of the phosphylated serine residue. This results in a charged phosphonate adduct that further repulses any nucleophile that attempts to come near and reactivate the enzyme. Pyridinium oximes like 2-PAM whose nucleophilic atom is found negatively charged in the body are rendered useless in attempting to attack the negatively charged residue. Therefore, in the current literature, there are no known therapies that are able to restore the aged enzyme back to its active form. The time it takes to age the enzyme varies for each nerve agent and is important to determine how fast someone would need treatment from an oxime before their AChE would be unable to be reactivated (Table 1.2). The aging half-time for VX is 48 hours, 14 hours for tabun, and a mere 2-6 minutes for soman[28]. The mechanism associated with aging has been studied and has been proven that upon dealkylation, it is actually the carbon-oxygen bond that is breaking, not the oxygen-phosphorus bond[29]. Soman ages the fastest due to it being able to form a stable carbocation once dealkylated via a methyl shift (Scheme 1.4). This also helps to

support the idea that water is not a good enough nucleophile to attack the phosphate group and can only attack the alkyl group once it "falls off" the enzyme.

Agent	Inhibition Rate (M^{-1} min^{-1})	Aging Rate (h^{-1})
GA	7.4×10^{6}	3.6×10^{-2}
GB	2.7×10^{7}	2.3×10^{-1}
GD	9.2×10^{7}	6.6
GF	4.9×10^{8}	9.9×10^{-2}
VX	1.2×10^{8}	1.9×10^{-2}
VR	4.4×10^{8}	5.0×10^{-3}

Table 1.2: Comparative inhibition and aging rates for common nerve agents on AChE[30]



Scheme 1.4: Proposed mechanism from literature for the formation of the aged enzyme via dealkylation via soman-inhibited AChE[31]

Once the enzyme is aged, it cannot be directly reactivated. Therefore, to restore enzyme activity of the aged AChE, it must be first realkylated (Figure 1.5). Only then could the inhibited enzyme be treated with therapeutic oximes to recover the activity of AChE. To prevent detrimental effects due to long-term exposure seen with reoccurring use of substances such as pesticides, the ability to realkylate the enzyme is vital. Many agricultural workers are exposed to non-lethal does of these agents, for which overtime the continued use can build up the toxins in their bodies, aging AChE over time, and can therefore cause severe long term damage. This has been seen often and is the cause of many of the organophosphorus toxicities seen today all across the globe.

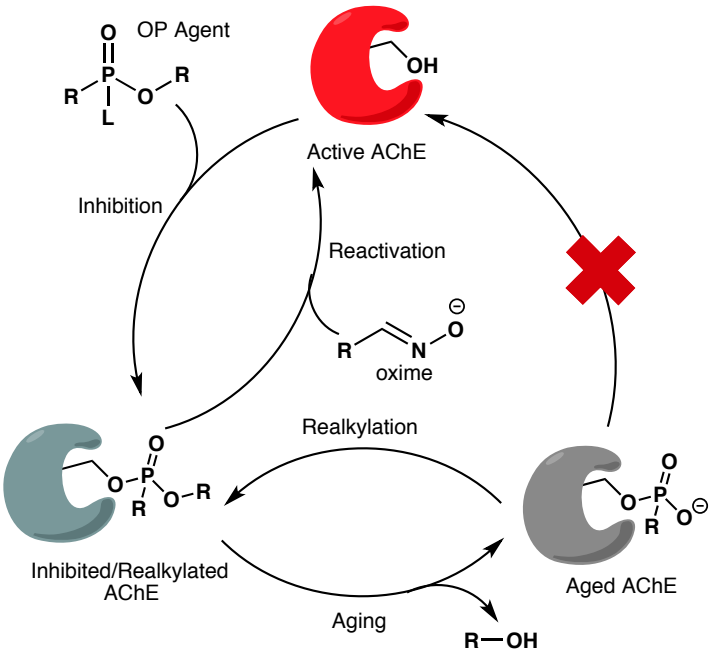
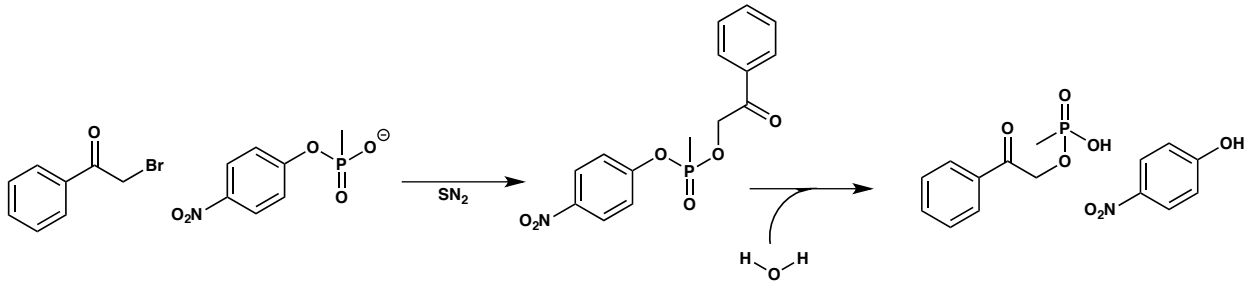


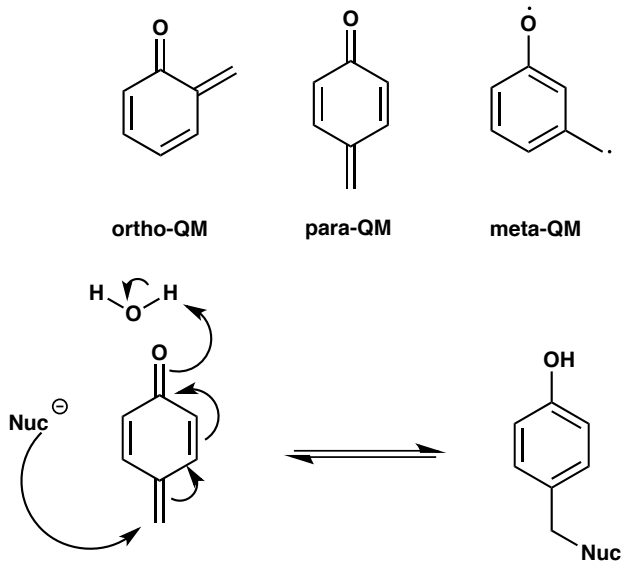
Figure 1.5: AChE potential inhibition and realkylation pathways

1.4 Quinone Methide Precursors as Realkylators of Aged AChE

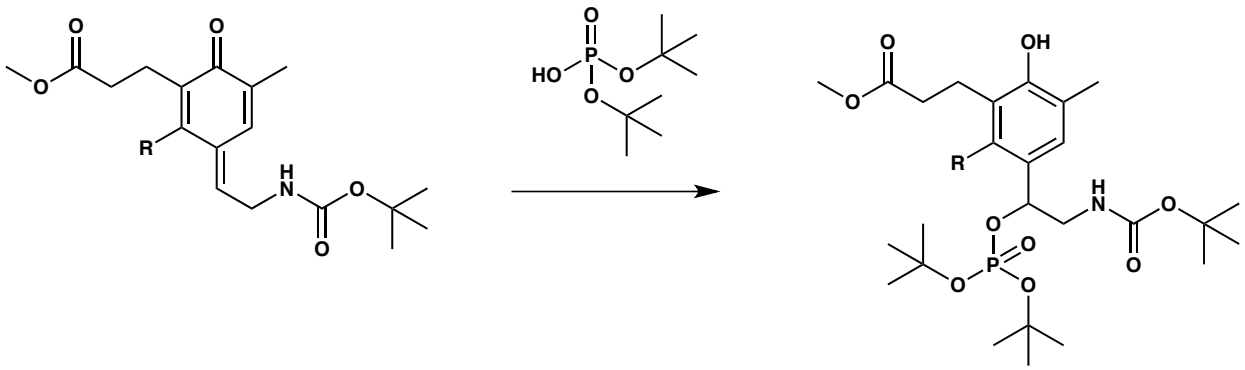
The idea of realkylating the enzyme as a treatment option was studied briefly in the 1960s and 1970s, but was abandoned due to unsuccessful attempts at regenerating activity and the lack of structural information about the enzyme AChE. This route to reversing enzyme toxicity is largely unexplored. Previous research studies have shown the use phenylacyl bromide as alkylators of phosphonates (Scheme 1.5). While it was able to alkylate the model (40% alkylation in less than 10 minutes), they were not able to resurrect aged AChE *in vitro*.[32] Therefore, it did not seem like just any electrophile could be a sufficient alkylator of AChE. Quinone methides came into the mix of potential realkylators as they were thought to be a suitable electrophile to realkylate the charged phosphylated residue. They are very reactive due to their ability to act as a Michael acceptor with the large driving force coming from returning the QM back to aromaticity (Scheme 1.6). Quinone methides (QM) have been shown to alkylate a variety of compounds, and they have even been shown to alkylate DNA[33]. Quinone methides have also been shown to realkylate phosphodiester bonds, which is why they have been of interest in this field for their potential to realkylate AChE (Scheme 1.7). It became our research group's interest to further study QMs.



Scheme 1.5: Phenylacyl bromide alkylators to realkylate phosphonates



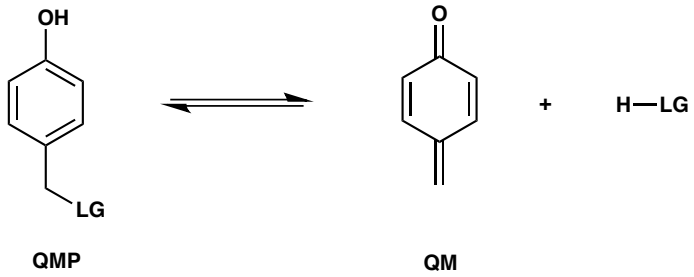
Scheme 1.6: Reaction of electrophilic quinone methides with nucleophiles



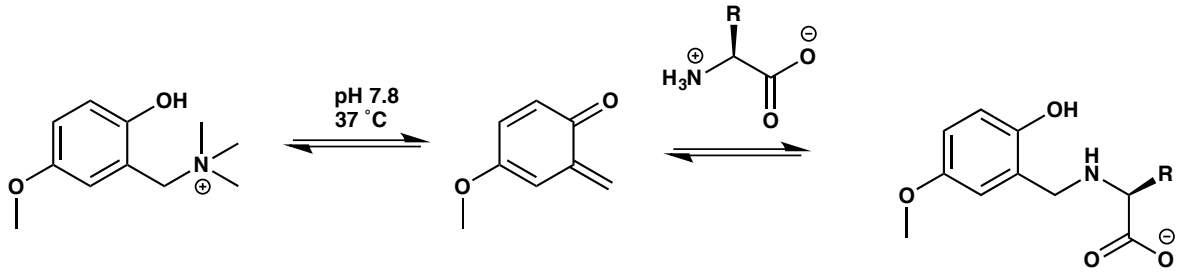
Scheme 1.7: Phosphate alkylation using a QM[34]

It is hypothesized that QMs could be the solution to realkylate the aged enzyme, as they have been seen to be able to alkylate phosphates already. In order to have potential in the body, a quinone methide precursor (QMP) would need to be used to prevent alkylation of DNA and other structures in the body, as this would cause new problems, like cancer, even if it did realkylate AChE. QMPs have a leaving group that in

the body could leave and allow for the formation of the desired QM in the active site of AChE (Scheme 1.8). QMPs have been used in other studies at physiological conditions to form QMs and go on to alkylate substances like amino acids (Scheme 1.9).



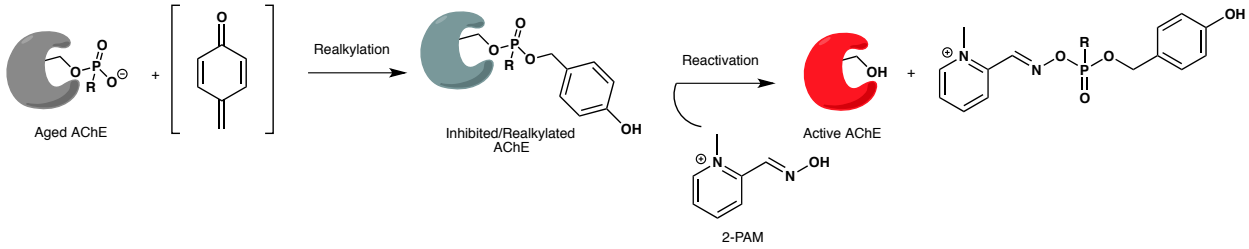
Scheme 1.8: QM formation from QMP



Scheme 1.9: QMP alkylation of an amino acid via elimination of a benzylammonium salt[35]

Realizing the vast capacity of QMPs to generate QMs under physiological conditions, their ability to react with poor nucleophiles including phosphodiesters, and the vast opportunity for tunability by varying substituents on their framework, this class of compounds were chosen for exploration in the realkylation of aged AChE (Scheme 1.10). By adding various electron withdrawing or donating groups to the ring, suppression and promotion of both leaving group ability and electrophilicity of the QM

are able to be fine tuned and adapted to be the right combination to affectively realkylate aged AChE.



Scheme 1.10: Use of QMs to realkylate aged AChE

Libraries of QMPs were designed after computational studies in order to narrow down the vast possibilities of QMPs. The general design followed similar structures to 2-PAM and acetylcholine in order to hopefully get adequate binding in the active site (Figure 1.6). The computations done in our research were used to guide our synthesis of QMPs. The computations determined the reactivity and docking pose of the QMPs in the active site of AChE.

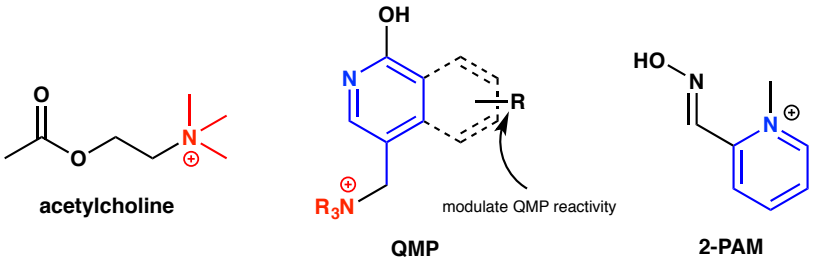


Figure 1.6: QMP with affinity for the enzyme active sites

Our current lead compound is a pyridine derivative with a pyrrolidine leaving group (Figure 1.7). This compound's ability to realkylate aged AChE will be discussed

later in this thesis. Computational studies have shown that quinoline and isoquinoline frameworks would fit better in the active site due to the favorable interactions with residues in AChE. The computational studies have shown to be very promising, so the next step logical step in the synthesis project was to make libraries of quinolines to see how they compare to the current lead compound and see if the increased favorable binding in the active site leads to more or less realkylation. A similar structure to the current lead was followed in making these derivatives; each was sought to have a hydroxyl group off the ring with the nitrogen, and an amine leaving group. Previous screenings showed that pyrrolidine, piperidine, 2-methylpyrrolidine, and diethyl amine as the leaving groups have the most activity in realkylating aged AChE, so those were the four amines used to generate the various libraries that will be shown in this thesis.

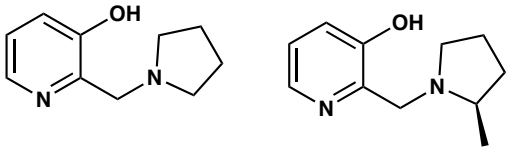


Figure 1.7: Our current lead compounds, 2-(pyrrolidin-1-ylmethyl)pyridin-3-ol and (*R*)-2-(2-methylpyrrolidin-1-yl)methyl)pyridin-3-ol, respectively

This thesis will cover the synthesis of various QMP frameworks and their subsequent libraries. It will also cover the evaluation of this novel class of potential realkylators in potentially restoring activity to aged AChE.

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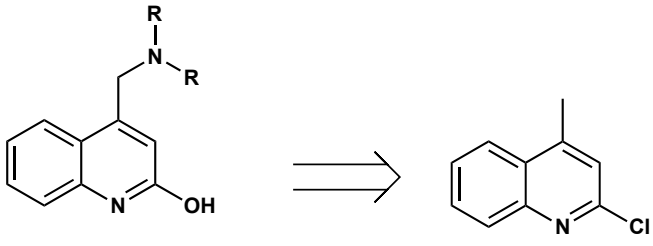
CHAPTER TWO

SYNTHESIS OF 2-HYDROXY-4-(AMNIOMETHYL)QUINOLINE

2.1 Introduction

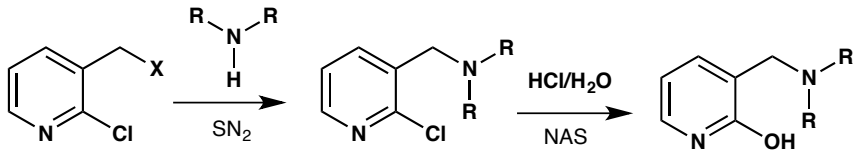
As discussed in the introduction, the goal of our research is to synthesis quinoline libraries with similar structures to the current lead compound (Figure 1.7). The libraries of quinolines desired must be able to form a quinone methide once in the body in order to have the desired effect in being a strong an selective electrophile to alkylate AChE. The problem is that in order to adequately make a library of compounds suitable for a drug that could go on to realkylate AChE, it would need to be cost effective as well, or else it would be hard to make a large enough library to screen. Drawbacks with quinoline frameworks include complicated syntheses to the substituted frameworks and expensive starting materials. Most of the substituted quinoline frameworks are commercial available.

A substituted quinoline found that was commercially available was 2-chloro-4-methylquinoline, and was thought this starting material could be converted into the desired framework if halogenation of the methyl substituent proved possible (Scheme 2.1). The starting material is readily available from reputable suppliers, but no di-halogenated versions were found available.



Scheme 2.1: Retrosynthesis of 2-hydroxy-4-(aminomethyl)quinoline from 2-chloro-4-methylquinoline

Our research group's synthesis routes often involve taking a di-halogenated compound and performing an S_N2 and a subsequent NAS reaction in order to get the desired substituents onto the ring (Scheme 2.2). This method has proven to be very efficient in generating libraries as it is very easy to then vary the amine leaving group placed onto the framework. The ability to generate the hydroxypyridine as the last step of the reaction is also helpful in the purification of these compounds, as it is easier to isolate these compounds when they are less polar. The ability to change the leaving group on these compounds is vital as our computational and kinetic studies have shown that varying the amine leaving group drastically affects the realkyating ability of these compounds on aged AChE.



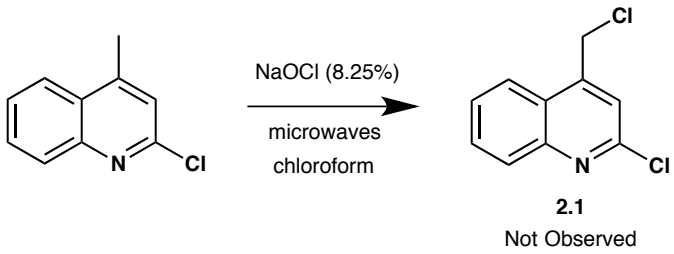
Scheme 2.2: Derivation of di-halogenated compounds to the desired libraries following an S_N2 and NAS reaction

This chapter will discuss the synthesis of a library of QMPs based on the general framework of 2-hydroxy-4-(aminomethyl)quinoline.

2.2 Results, Discussion, and Conclusions

The di-halogentated species was attempted to be synthesized from 2-chloro-4-methylquinoline by utilizing common household bleach and a microwave. According to the literature from 1998, common household bleach was found effective in rapidly

chlorinating side chains of heterocyclic compounds with the use of microwave radiation[1]. However, there is no yield reported in the literature for this reaction, only chemical shifts of the isolated product. When the procedure was replicated in a microwave at 2450 MHz for 2.5 minutes, **2.1** was not observed (Scheme 2.3). The solvent varied from chloroform to water, but still no product formation occurred. The microwave settings were adapted to 110 ˚C at 220 W for 10 cycles of 1 minute heating and two minutes cooling, but still **2.1** was not observed.

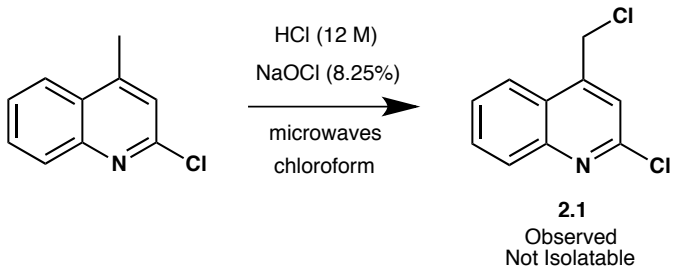


2.1
Not Observed

Scheme 2.3: Attempted synthesis of **2.1** using microwave irradiation and bleach

According to the polymer literature, a similar reaction with bleach can be performed to chlorinate polymers but they emphasized the importance of maintaining the pH at 8.4 during the reaction[2]. Hydrochloric acid (12 M) was added until this pH was reached. According to the literature, this reaction occurred at ambient temperature and there was no need for a microwave. After no formation of **2.1** was observed at room temperature or at reflux, the same reaction shown in Scheme 2.3 was performed with the addition of hydrochloric acid (Scheme 2.4). Product formation was observed by TLC, but was not able to be isolated, as there were also 10+ other side products observed for the reaction. While the product was formed, the conditions were not

sufficient to generate enough material of **2.1** to create a library of QMPs. The temperature of the microwave, the time, and the amount of hydrochloric acid added were all varied to see if this could cause more product formation, but all seemed to not be responsible for the reason why the reaction was low-yielding.

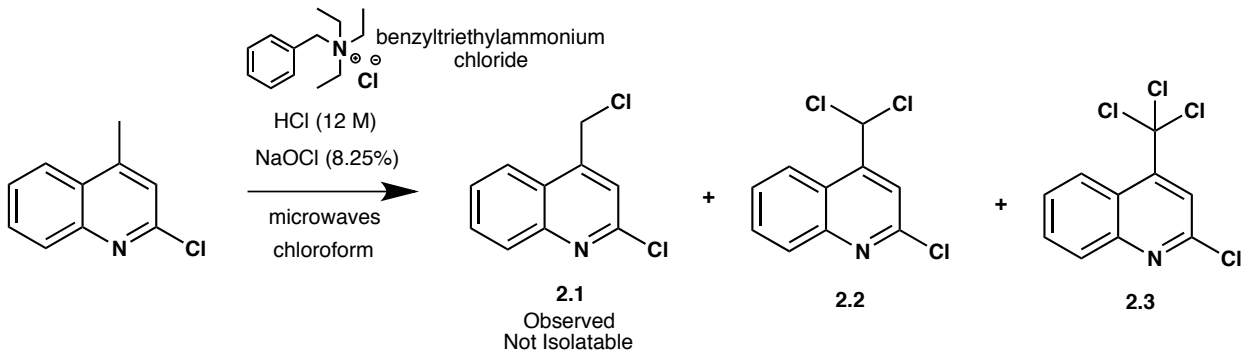


2.1
Observed
Not Isolatable

Scheme 2.4: Attempted synthesis of **2.1** with the addition of concentrated hydrochloric acid

The same literature source that cited the use of hydrochloric acid also cited the potential in using benzyltriethylammonium chloride, a suitable phase transfer catalyst[2]. According to the literature, the reaction is possible without this catalyst to chlorinate the side chain, but the use of it speeds up the reaction. The hope was that by using this catalyst, more product could be formed under similar reaction conditions and would allow **2.1** to be isolated (Scheme 2.5). What was discovered was the catalyst seemed to overhalogenate under these conditions. TLC analysis of the reaction proved product formation of **2.1**, plus the formation of two other compounds with similar R_f values to it and the starting material. Upon mass spectrometric analysis of the reaction mixture, it was seen that the mono-chlorinated starting material still remained, but three products were generated in the reaction. Not only was the desired di-chlorinated product **2.1**

observed, the tri-chlorinated **2.2** and tetra-chlorinated **2.3** products were observed as well (Figures 2.1 and 2.2).



Scheme 2.5: Attempted synthesis of **2.1** using a phase transfer catalyst

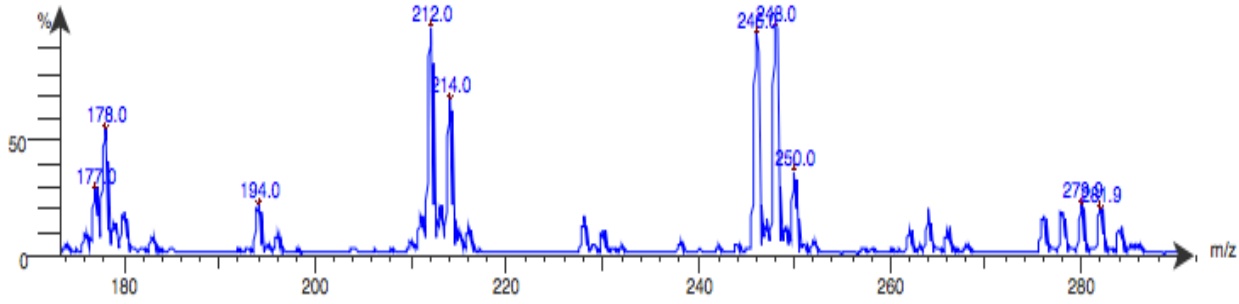


Figure 2.1: Mass spectrometric data from the reaction in Scheme 1.5; Exact mass 2-chloro-4-methylquinoline: 177, **2.1:** 211, **2.2:** 244, **2.3:** 278

The microwave conditions proved to be too harsh with the phase transfer catalyst, as there was no way of removing the starting material without forming byproducts **2.2** and **2.3**. The conditions were reattempted out of the microwave at ambient temperature, varying the time allowed to react. Regardless if it was two hours or twenty-four hours, the same array of products was observed for the reaction. It

seemed that the phase transfer catalyst favored the formation of the tetra-chlorinated product **2.3**, and it would not allow for the sole formation of product **2.1**. The microwave chlorination conditions to generate the desired product were therefore deemed to be inconsequential to make enough of the compound to use to generate a library of QMPs in a decent yield. Other reaction conditions were sought to generate a di-halogenated compound to use for the QMP library.

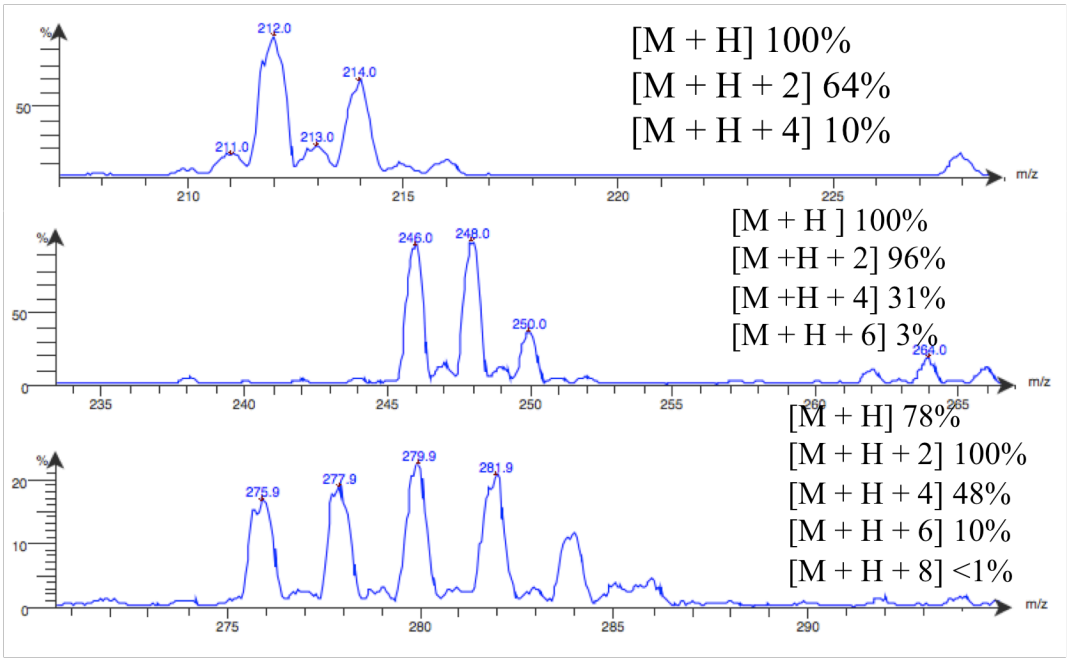


Figure 2.2: Zoomed in mass spectrometric data for **2.1**, **2.2**, and **2.3**, respectively, with the respective ratios of the chlorine isotopes included

It was stated in the literature that the mechanism of the chlorination reactions involved radicals. The propagation step in radical chlorination is known to be exothermic. This is due to the strong H-Cl bond (BDE = 103 kcal/mol)[3], which brings the reactant energy very close to the transition state energy, so the activation energy is not very high. For radical bromination on the other hand, the propagation step is known to

be endothermic. This is due to a weaker H-Br bond (BDE = 87 kcal/mol)[3], which puts its reactants at a much lower energy than the transition state, so there is a high activation energy to form the products (Figure 2.3).

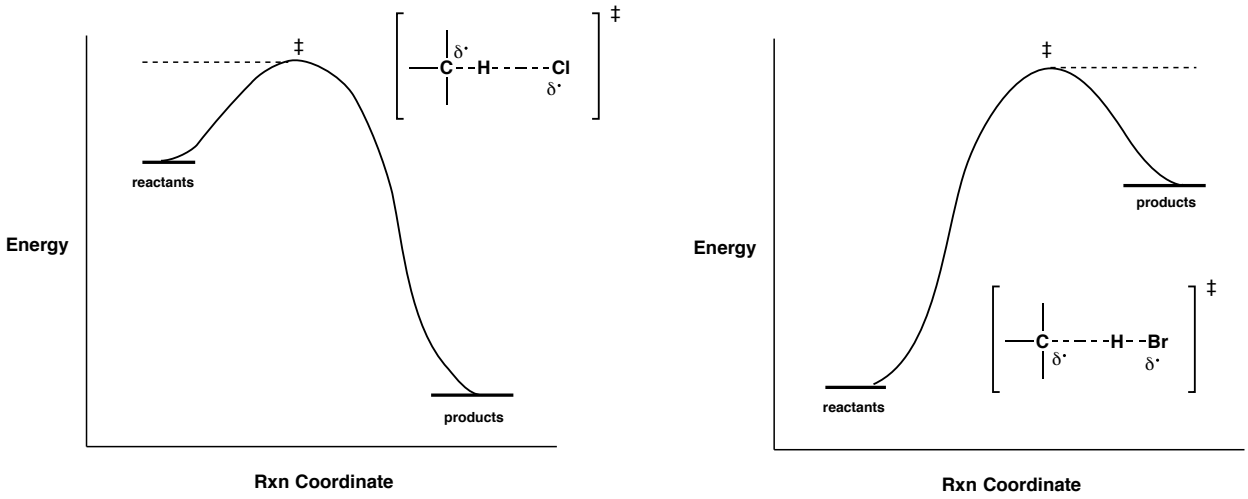
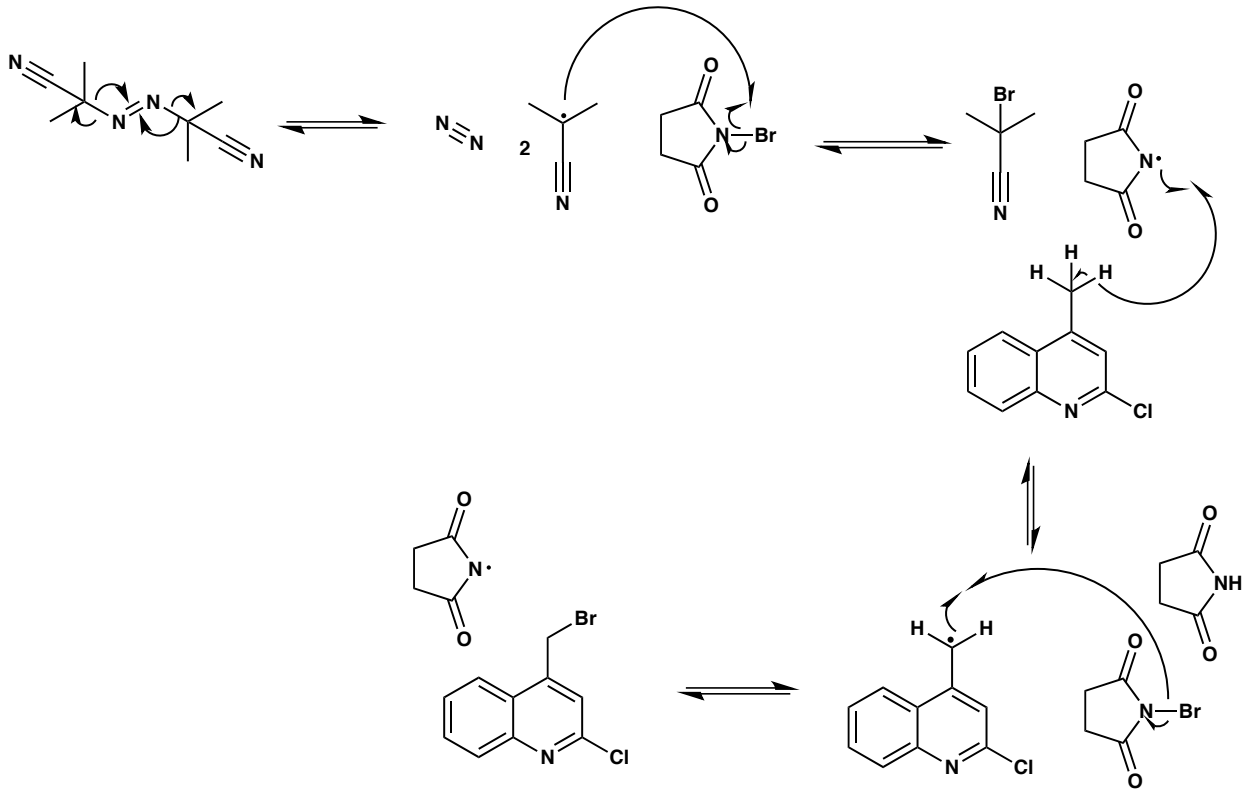


Figure 2.3: Reaction coordinate diagrams for chlorination and bromination, respectively

Since chlorination itself is exothermic, it could explain why it is being observed to not only add once, but twice or three times. Not as much energy is required to chlorinate and overcome the activation barrier so it is easy to believe that under ambient conditions or in the microwave it could overchlorinate the product since it has enough energy to form the undesired products of **2.2** and **2.3**. Since the radical bromination reaction is endothermic for most C-H bonds, it would be expected that it is harder to di- and tri- brominate 2-chloro-4-methylquinoline since it would require more energy to overcome the large energy barrier. Bromination conditions were therefore sought out to generate solely the mono-brominated product that could be used to synthesis the libraries of QMPs.

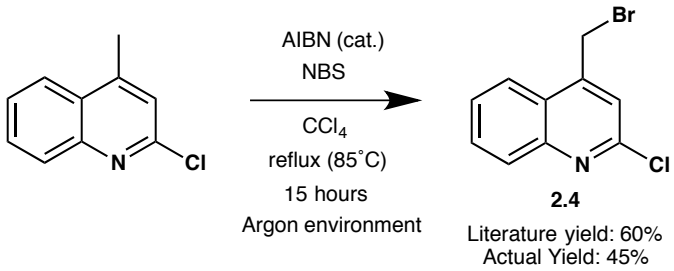
According to the literature, *N*-bromosuccinimide (NBS) has been shown to brominate side-chains of various pyridine and quinoline heterocycles in decent yields with the use of azobisisobutyronitrile (AIBN) as a radical initiator[4]. A proposed mechanism for the reaction with 2-chloro-4-methylquinoline is shown in Scheme 2.6.



Scheme 2.6: Proposed radical mechanism to brominate the methyl group using AIBN and NBS

By using a brominating agent rather than a chlorinating agent, the hope was that the product would be solely mono-brominated. However, disappointingly it was observed that after a certain period of time, di- and tri-brominated products are formed in significant quantities. Even when the di- and tri-brominated products form, the starting material is still present as well. To avoid having to worry about separating out multiple

brominated compounds, the reaction was monitored by TLC to determine when these byproducts started to form. It seemed that after 16 hours, polybrominated products began to form. The time to produce additional brominated products was a lot longer for this reaction compared to the previous chlorination conditions discussed, so this procedure was attempted to be optimized to obtain the framework needed. The reaction was stopped after 15 hours so that only the starting material and **2.4** were isolated (Scheme 2.7).



Scheme 2.7: Synthesis of **2.4** from 2-chloro-4-methylquinoline

It is important to note that the R_f values for 2-bromo-4-methylquinoline and **2.4** are 0.40 and 0.34 in 10:1 hexanes: ethyl acetate, respectively. Therefore, it proved very difficult to separate the two products. With use of flash chromatography and a finely controlled gradient, the two materials could be separated from each other, though losing a small amount of both in mixed fractions, which explains the low yield of 45% compared to the literature yield of 60%. However, due to the inexpensiveness of the starting material, this yield proved to be suitable as it was performed on a gram scale and was sufficient to generate enough product to synthesize a library of QMPs with. A mass spec was ran on the final compound, and the expected ratios for both bromine

and chlorine isotopes were observed (Figure 2.4), helping to prove the identity of the compound and the validity of this reaction.

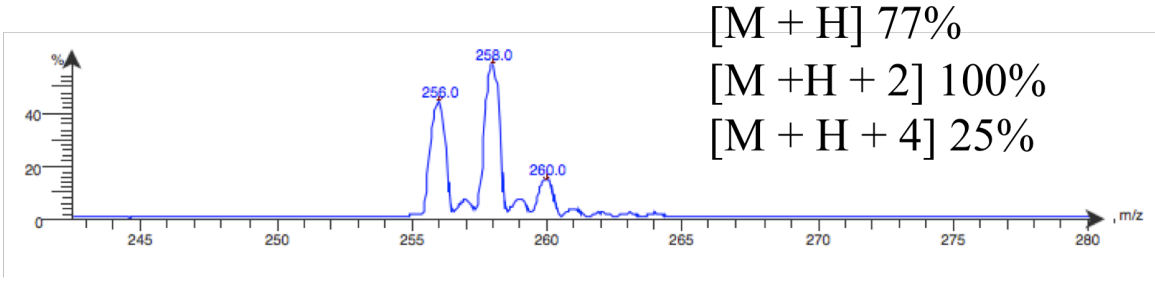
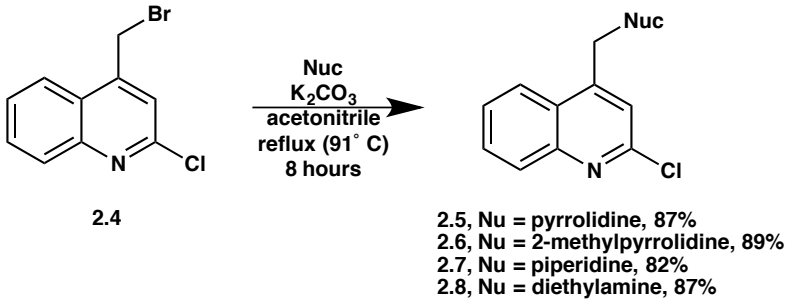


Figure 2.4: Zoomed in mass spectrometric data for **2.4** with the respective ratios of the bromine and chlorine isotopes included; Exact mass **2.4:** 255

With di-halogenated compound in hand, the general procedure in Scheme 2.2 was followed to generate the library for this framework. The amines utilized for the library of compounds were pyrrolidine, 2-methylamine, piperidine, and diethylamine. These amines have shown promise on our lead compound's framework as being the best amines in realkylating aged AChE in our kinetics studies. Other amines and nucleophiles could be added to further build upon the library of this framework, but for initial kinetic testing only four were made to see if the framework has any activity (Scheme 2.8).

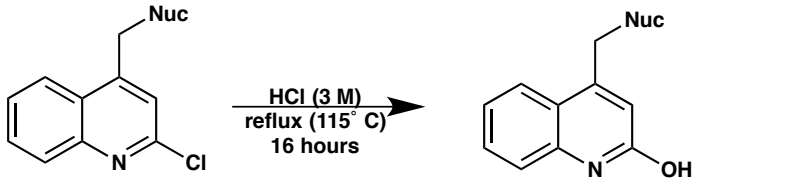


2.4

2.5, Nu = pyrrolidine, 87%
2.6, Nu = 2-methylpyrrolidine, 89%
2.7, Nu = piperidine, 82%
2.8, Nu = diethylamine, 87%

Scheme 2.8: S_N2 reaction of **2.4** and various amine nucleophiles

The yields for the S_N2 reaction were consistent with high yields around 80-90%. Once the amine was placed onto the framework, an NAS reaction was performed to exchange the chlorine on the ring with a hydroxyl group (Scheme 2.9). The yields for this reaction varied greatly, from ~40-70%. Some of the material seems to get lost on the column used to purify the final product, which can explain the yield and could potentially be optimized. This was the final step to making the desired quinone methide precursor that will go on to kinetic testing to see its ability to realkylate aged AChE and restore activity in the enzyme. The kinetic results of compounds **2.9-2.12** will be discussed later on in this thesis.



2.5-2.8

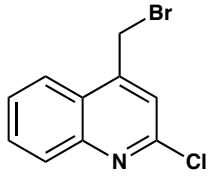
2.9, Nu = pyrrolidine, 74%
2.10, Nu = 2-methylpyrrolidine, 56%
2.11, Nu = piperidine, 37%
2.12, Nu = diethylamine, 38%

Scheme 2.9: NAS reaction of **2.5-2.8** with 3 M hydrochloric acid to build final QMP compounds for testing, **2.9-2.12**

This method of bromination can prove synthetically useful in generating other frameworks to make QMPs. The use of NBS and AIBN to halogenate side chains proved to be highly useful in our research for the best halogen source. This method provided and inexpensive and semi-efficient way to synthesize a library of 2-hydroxy-4-(aminomethyl)quinoline compounds.

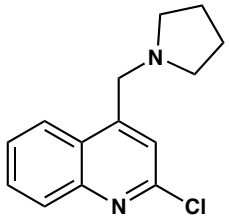
2.3 Experimental Data

General Methods The solvents used in these experiments were not purified any further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



2.4

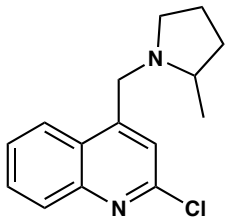
4-(bromomethyl)-2-chloroquinoline (2.4) To a solution of CCl_4 (10 mL) and 2-chloro-4-methylquinoline (1.00 g, 5.63 mmol) was added AIBN (0.277 g, 1.69 mmol) and *N*-bromosuccinimide (1.10 g, 6.19 mmol) . The reaction mixture was refluxed for 15 h under nitrogen atmosphere. The insoluble solids were removed via vacuum filtration and the filtrate was concentrated. The resulting crude product was purified by chromatography (10:1 hexanes:EtOAc) to yield **2.4** (0.644 g, 45%) as a white powdery solid: R_f 0.39 (10:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.13–8.06 (m, 2 H), 7.78 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.66 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.45 (s, 1 H), 4.80 (s, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.36, 148.41, 145.48, 130.87, 129.55, 127.41, 124.75, 123.42, 122.46, 27.13; HRMS (ESI) calcd for (M+Na) $C_{10}H_7BrClN$: 277.9343, found 277.9310.



2.5

2-chloro-4-(pyrrolidin-1-ylmethyl)quinoline (2.5) To a solution of acetonitrile (10 mL), **2.4** (2.50 mmol), and potassium carbonate (0.347 g, 2.50 mmol) was added pyrrolidine (0.42 mL, 5.02 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **2.5** (0.644 g, 45%) as a yellow oil: R_f 0.86 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.18 (dd, 1 H, *J* = 0.8,

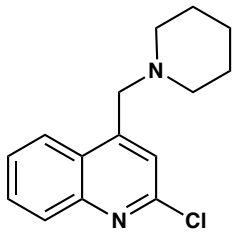
8.4 Hz), 8.04 (dd, 2 H, *J* = 0.8, 8.4), 7.74 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.59 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.47 (s, 1 H), 4.06 (s, 2 H), 2.69–2.55 (m, 4 H), 1.89–1.79 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.87, 148.82, 148.03, 130.13, 129.12, 126.63, 126.10, 123.92, 121.52, 56.75, 54.59, 23.74; HRMS (ESI) calcd for (M+H) $C_{14}H_{15}ClN_2$: 247.7455, found 247.0991.



2.6

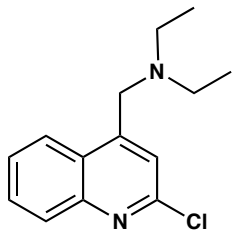
2-chloro-4-((2-methylpyrrolidin-1-yl)methyl)quinoline (2.6) To a solution of acetonitrile (10 mL), **2.4** (0.451 g, 1.76 mmol), and potassium carbonate (0.243 g, 1.76 mmol) was added pyrrolidine (0.36 mL, 3.52 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **2.6** (0.409 g, 89%) as a yellow oil: R_f 0.88 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.20 (dd, 1 H, *J* = 0.8, 8.4 Hz), 8.03 (dd, 2 H, *J* = 0.8, 8.4), 7.71 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.57 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.49 (s, 1 H), 4.41 (d, 1 H, *J* = 14.4) 3.56 (d, 1 H, *J* = 14.4), 2.96–2.90 (m, 1 H), 2.61–2.54 (m, 1 H), 2.16 (q, 1 H, *J* = 8.8), 2.06–1.95 (m, 1 H), 1.84–1.64 (m, 2 H), 1.56–1.44 (m, 1 H), 1.23 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.84, 149.52, 148.04, 130.11, 129.10, 126.57, 126.18, 123.99, 121.73,

60.56, 54.94, 54.68, 32.82, 21.84, 19.27; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1080, found 261.1146.



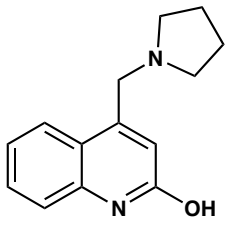
2.7

2-chloro-4-(piperidine1-ylmethyl)quinoline (2.7) To a solution of acetonitrile (10 mL), **2.4** (0.396 g, 1.54 mmol), and potassium carbonate (0.213 g, 1.54 mmol) was added pyrrolidine (0.30 mL, 3.08 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **2.7** (0.328 g, 82%) as a light yellow oil: R_f 0.40 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.18 (dd, 1 H, *J* = 0.8, 8.4 Hz), 8.03 (dd, 2 H, *J* = 0.8, 8.4), 7.71 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.55 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.47 (s, 1 H), 3.84 (s, 2 H), 2.56–2.44 (m, 4 H), 1.67–1.56 (m, 4 H), 1.52–1.42 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.81, 148.45, 148.09, 130.11, 129.09, 126.51, 126.40, 124.10, 121.77, 59.85, 55.06, 26.05, 24.21; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1153, found 261.1213.



2.8

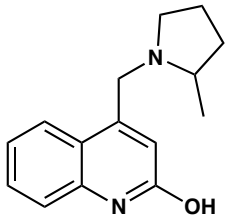
***N*-((2-chloro-quinoline-4-yl)methyl)-*N*-ethylethanamine (2.8)** To a solution of acetonitrile (10 mL), **2.4** (0.556 g, 2.17 mmol), and potassium carbonate (0.300 g, 2.17 mmol) was added pyrrolidine (0.45 mL, 4.34 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **2.8** (0.471 g, 87%) as a yellow oil: R_f 0.53 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.18 (dd, 1 H, *J* = 0.8, 8.4 Hz), 8.03 (dd, 2 H, *J* = 0.8, 8.4), 7.71 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.55 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.52 (s, 1 H), 3.97 (s, 2 H), 2.61 (q, 4 H, *J* = 6.8 Hz), 1.08 (t, 6 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.99, 149.89, 148.02, 130.07, 129.14, 126.49, 126.23, 123.77, 121.68, 54.44, 47.59, 11.92; HRMS (ESI) calcd for (M+H) $C_{14}H_{17}ClN_2$: 249.1153, found 249.1192.



2.9

4-((2-methylpyrrolidin-1-yl)methyl)quinolin-2-ol (2.9) To a solution of hydrochloric acid (3 M, 5 mL) was added **2.5** (0.250 g, 1.01 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by

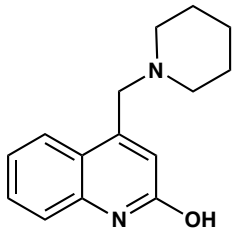
chromatography (1:1 hexanes:EtOAc) to yield **2.9** (0.172 g, 74%) as a off-white powdery solid: R_f 0.78 (1:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.93 (dd, 1 H, *J* = 0.8, 8.0 Hz), 7.49 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.34 (dd, 2 H, *J* = 0.8, 8.0), 7.22 (td, 1 H, *J* = 1.2, 8.4 Hz), 6.78 (s, 1 H), 3.84 (s, 2 H), 2.68–2.56 (m, 4 H), 1.89–1.79 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 163.84, 149.97, 138.34, 130.35, 124.83, 122.44, 120.21, 119.63, 116.12, 57.47, 54.48, 23.73; HRMS (ESI) calcd for (M+H) $C_{14}H_{16}N_2O$: 229.1335, found 229.1498.



2.10

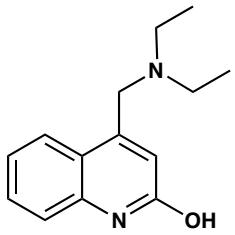
4-((2-methylpyrrolidin-1-yl)methyl)quinolin-2-ol (2.10) To a solution of hydrochloric acid (3 M, 5 mL) was added **2.6** (0.250 g, 0.961 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **2.10** (0.130 g, 56%) as a off-white powdery solid: R_f 0.78 (1:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.99 (dd, 1 H, *J* = 0.8, 8.0 Hz), 7.50 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.37 (dd, 2 H, *J* = 0.8, 8.0), 7.22 (td, 1 H, *J* = 1.2, 8.4 Hz), 6.83 (s, 1 H), 4.25 (d, 1 H, *J* = 14.4) 3.34 (d, 1 H, *J* = 14.4), 3.04–2.94 (m, 1 H), 2.61–2.50 (m, 1 H), 2.15 (q, 1 H, *J* = 8.8), 2.04–1.92 (m, 1 H),

1.80–1.59 (m, 2 H), 1.54–1.41 (m, 1 H), 1.22 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 163.99, 150.67, 138.36, 130.32, 124.88, 122.39, 120.37, 119.71, 116.17, 60.39, 55.63, 54.56, 32.89, 21.85, 19.30; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1504.



2.11

4-(piperidin-1-yl)methyl)quinolin-2-ol (2.11) To a solution of hydrochloric acid (3 M, 5 mL) was added **2.7** (0.250 g, 0.959 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **2.11** (0.086 g, 37%) as a white powdery solid: R_f 0.?? (1:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.95 (dd, 1 H, *J* = 0.8, 8.0 Hz), 7.49 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.36 (dd, 2 H, *J* = 0.8, 8.0), 7.22 (td, 1 H, *J* = 1.2, 8.4 Hz), 6.80 (s, 1 H), 3.66 (s, 2 H), 2.57–2.41 (m, 4 H), 1.66–1.54 (m, 4 H), 1.51–1.39 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 163.95, 149.45, 138.43, 130.31, 124.93, 122.32, 120.46, 119.81, 116.19, 60.56, 55.01, 26.08, 24.28; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1502.



2.12

4-((diethylamino)methyl)quinolin-2-ol (2.12) To a solution of hydrochloric acid (3 M, 5 mL) was added **2.8** (0.250 g, 1.00 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **2.12** (0.089 g, 38%) as a light yellow powdery solid: R_f 0.?? (1:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.95 (dd, 1 H, *J* = 0.8, 8.0 Hz), 7.40 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.38 (dd, 2 H, *J* = 0.8, 8.0), 7.20 (td, 1 H, *J* = 1.2, 8.4 Hz), 6.88 (s, 1 H), 3.77 (s, 2 H), 2.59 (q, 4 H, *J* = 7.2 Hz), 1.06 (t, 6 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 164.19, 150.84, 138.42, 130.30, 124.66, 122.31, 120.40, 119.75, 116.31, 55.17, 47.41, 11.82; HRMS (ESI) calcd for (M+H) $C_{14}H_{18}N_2O$: 231.1492, found 231.1505.

2.4 References for Chapter 2

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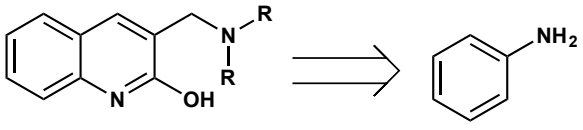
CHAPTER THREE

SYNTHESIS OF 2-HYDROXY-3-(AMNIOMETHYL)QUINOLINE

3.1 Introduction

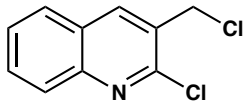
With such a malleable framework like quinoline, there is an abundance of varied combinations of placements for the leaving group on the fused ring system, as well as the hydroxyl group. Computationally, many combinations have shown promise in realklyating aged AChE. Since the 2-hydroxy-4-(aminomethyl)quinoline was obtained in the previous chapter, the next step was to see how changing the leaving group position from four to three affects its leaving group ability and its ability to resurrect the enzyme.

Unfortunately, as previously discussed, subsituted quinoline compounds are difficult to source, and the ones that are available are too expensive to use to generate a library. There was no commercial available quinoline that would allow for simple access to our desired framework. In order to have enough compound to make the QMPs and be cost effective, syntheses from small starting materials were sought that could to be used to produce the substituted quinoline frameworks. Literature reports suggested that aniline could provide access to the correct framework (Scheme 3.1). Our goal was to find a way to synthesize the di-halogenated compound framework with varying substitution to that discussed in the previous chapter.



Scheme 3.1: Retrosynthesis of 2-hydroxy-3-(aminomethyl)quinoline from aniline

Once generated with two halogens, an S_N2 and NAS could be performed to make the desired QMP libraries, like in Scheme 2.2. By having the ability to perform an S_N2, it enables us to vary the nucleophiles that can be put onto the QMP to serve as the leaving group to generate the QM. The ability to change up the leaving group on these compounds is imperative; being able to screen a variety of different leaving groups can help us hone in on what makes a compound a better or worse realkylator of AChE. Also, by being able to perform the NAS reaction last, it allows the isolation and purification of these compounds to be unproblematic as it is easily separated from the polar side products seen in some of these reactions.



3.1

Figure 3.1: Desired dihalogenated quinoline to generate a QMP library

This chapter will discuss the synthesis of a library of QMPs based on the general framework of 2-hydroxy-3-(aminomethyl)quinoline.

3.2 Results, Discussion, and Conclusions

Aniline is a common reagent used to generate quinoline the substituted quinoline framework (Figure 3.2). Common syntheses for quinoline with aniline involve the use of a variety of metals to catalyze the reaction, like copper and palladium. The literature has shown many successful reactions with these catalysts, but unfortunately all of the products tend to only be able to add aromatic carbons, like aryl groups, to the quinoline

framework. This would increase the difficulty in derivatizing these into our desired compounds. Other reaction conditions besides metal-catalysis were sought out that would be able to put useful substituents on the quinoline ring to use to make the QMP library with.

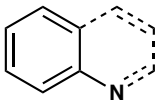
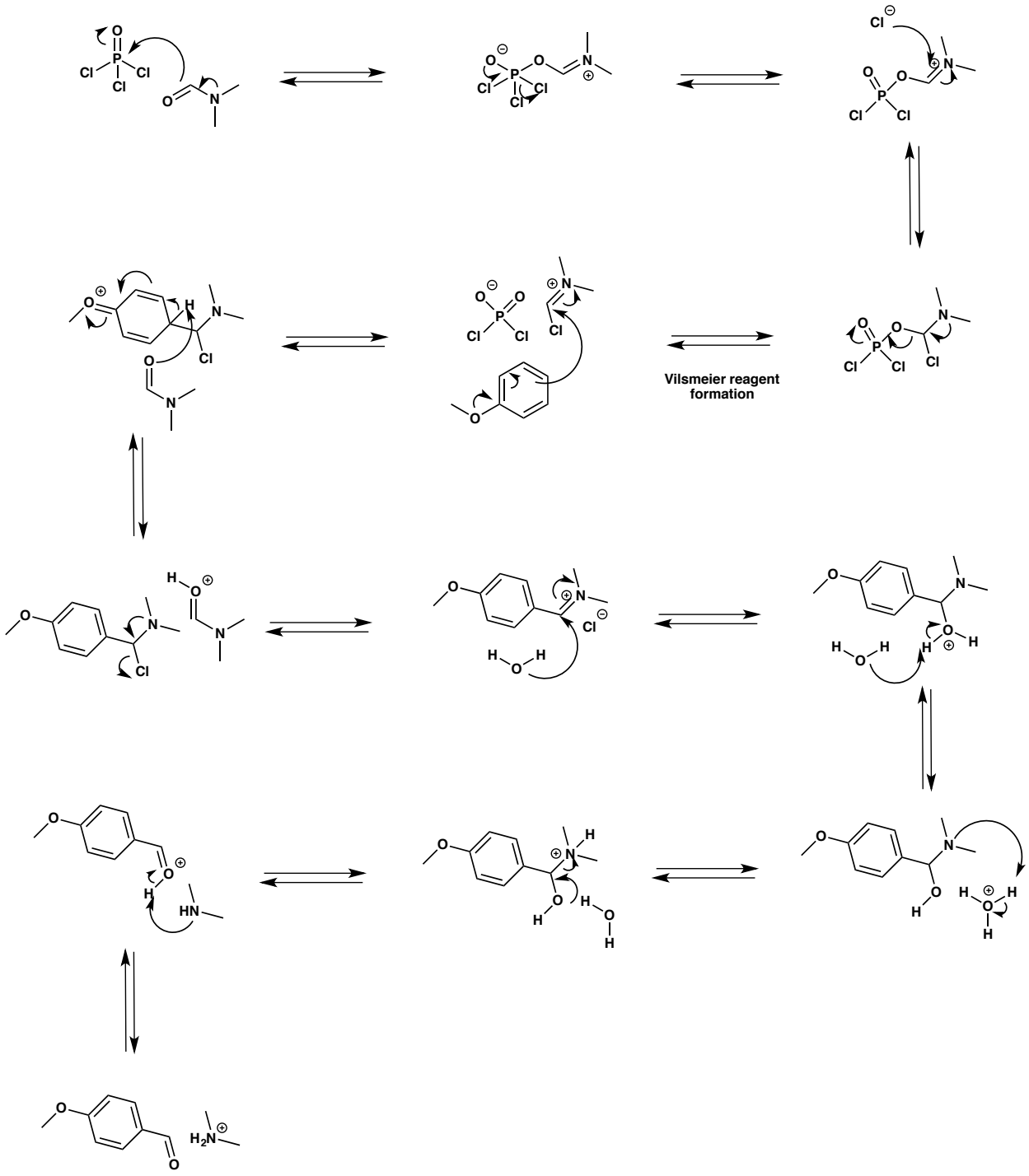
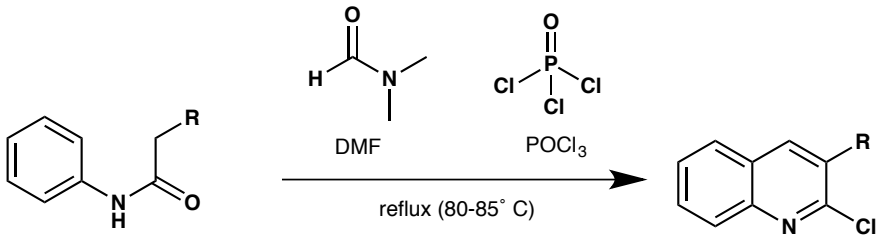


Figure 3.2: Use of aniline to generate quinoline framework

A synthesis was discovered in the literature that began with aniline and was used to make the desired **3.1** framework without the use of metals for catalysis[1]. The reaction relied on the Vilsmer-Haack reaction as vital to the synthesis of these frameworks. The Vilsmer-Haack reaction is an organic reaction that is typically used to convert an electron rich aromatic ring to an aryl aldehyde using DMF, $POCl_3$, and an aqueous work up[2]. DMF and $POCl_3$ form an iminium salt, commonly known as the "Vilsmeier reagent". The aryl ring is then able to attack the iminium ion, rearomatize, and then form another iminium ion with the loss of chlorine, which with an aqueous workup gets converted to the aldehyde species (Scheme 3.2). This reaction is known to work well in generating aldehyde species onto aromatic compounds. According to the literature, aldehyde additions under these conditions are highly favored. However, the cited source uses the Vilsmeier reagent in a new way, using the formation of aromaticity rather than the formation of an aldehyde to drive the reaction to completion[1] (Scheme 3.3).

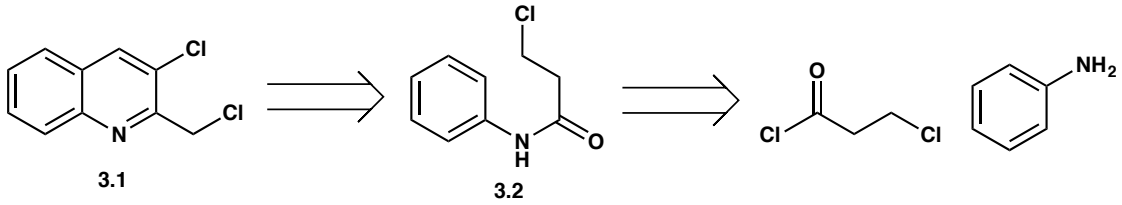


Scheme 3.2: Mechanism for the typical Vilsmeier-Haack formylation reaction to add an aldehyde to an aryl ring (anisole)



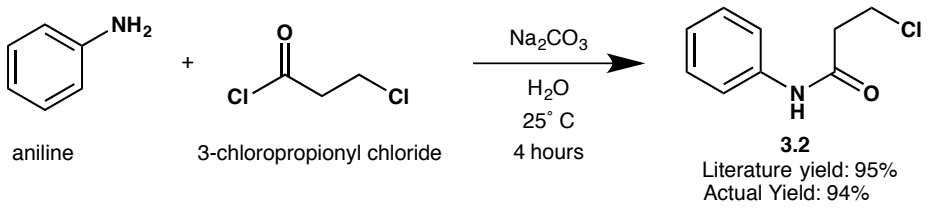
Scheme 3.3: Proposed reaction to generate quinoline framework from an amide

According to Scheme 3.3, the quinoline framework can be edited based on which amide compound is used in this reaction. By altering the R group, different substituents can be placed onto the ring structure. As stated above, the atomic difference between aniline and quinoline is three carbons. The Vilsmeier reagent, as shown in Scheme 3.2, is the source of one of the carbons. In order to get the desired **3.1** quinoline framework to make the libraries of QMPs, the R group shown in Scheme 3.3 would need to contain another carbon and another chlorine atom. This would generate the desired **3.1** product. In order to use the desired amide to use in this Vilsmeier-Haack reaction, it needs to be synthesized so that it would have the correct groups of the carbonyl. Aniline and 3-chloropropionyl chloride were thought to be the key starting materials to make the desired amide, **3.2**.

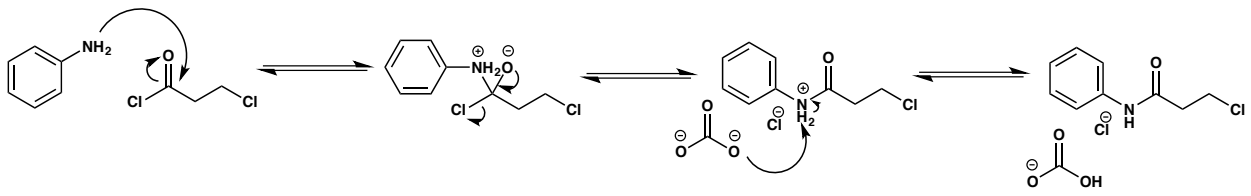


Scheme 3.4: Retrosynthetic analysis of the formation of **3.1** from aniline and 3-chloropropionyl chloride

The first part of the reaction therefore involves the formation of **3.2** from aniline and 3-chloropropionyl chloride through an amidation reaction (Scheme 3.5). The amine comes in to attack the very electron deficient acyl chloride and form the key tetrahedral intermediate. The chlorine is then eliminated to form a very stable carboxylic amide followed by a deprotonation (Scheme 3.6). This simple reaction is highly useful due to its addition of the three missing carbons discussed earlier that are necessary to form the quinoline framework. The R group being chlormethyl is necessary so that there is a suitable leaving group for the next step of the reaction. Depending on what substituents are desired on the final quinoline ring, though, this same reaction could be performed with varying acyl chlorides that could go on to manipulate the final quinoline framework.



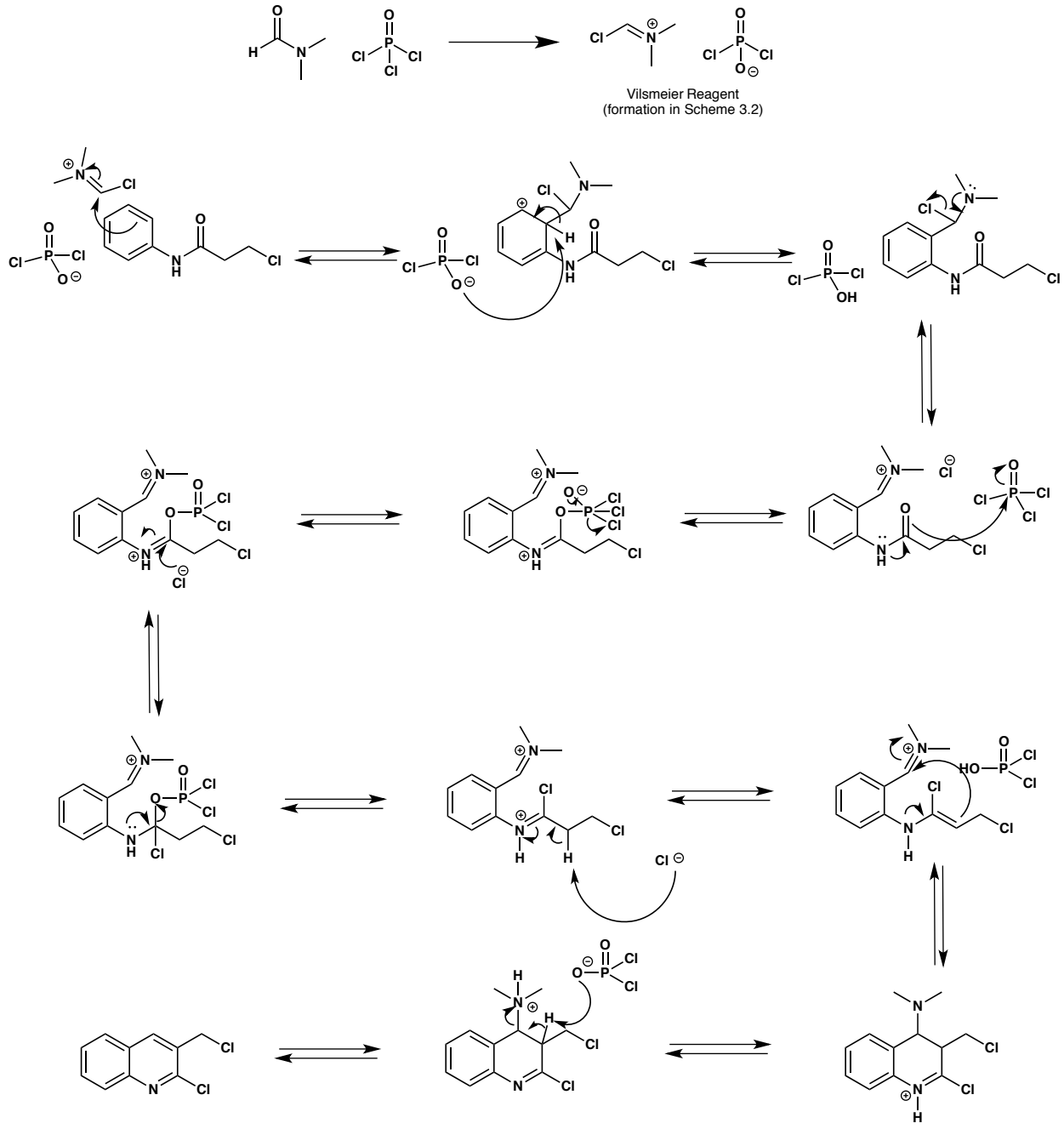
Scheme 3.5: Synthesis of **3.2** from aniline and 3-chloropropionyl chloride



Scheme 3.6: Mechanism for formation of **3.2** from aniline and 3-propionyl chloride

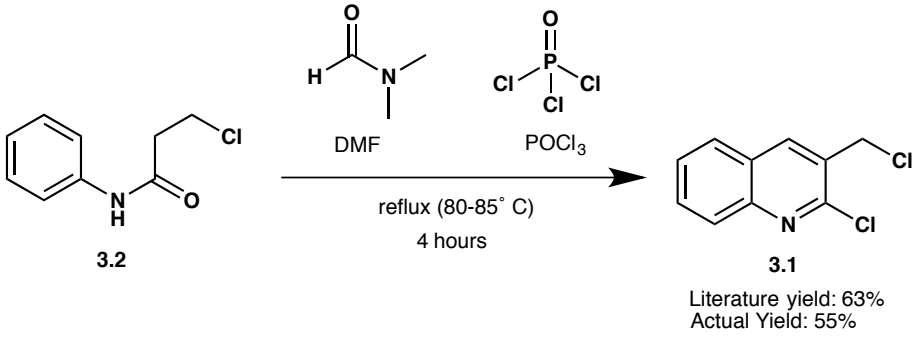
The next part of the reaction involves using **3.2** to generate the quinoline with the Vilsmeier-Haack Reaction. Once the Vilsmeier reagent is generated *in situ*, **3.2** can be

added to the reaction flask. A similar EAS reaction as the one shown in Scheme 3.2 occurs, except that after the second iminium ion forms, a series of steps occur with the aid of $POCl_3$ to close the second ring and form aromaticity (Scheme 3.7).



Scheme 3.7: Proposed mechanism for Vilsmeier-Haack quinoline formation

It is important that the Vilsmeier reagent reacts *ortho* to the amide group on the aryl ring in order to be in the correct placement to form the quinoline ring. Though the *para* position is favored due to steric effects, this would not be able to form the quinoline product, but could explain some of the side products seen in the reaction. Since the reaction is so similar to the one shown in Scheme 3.2, it is clear that adding water to the system would ruin the reaction and could cause side products containing an aldehyde to occur. Therefore, it is vital that this reaction is run in dry conditions and that no water is introduced into the system until the reaction is complete. As seen in the mechanism, the R group played no part in the overall reaction, which serves as proof as to why that group can be variable whereas the other ones cannot. The amide intermediate used from Scheme 3.5 proved to be a suitable choice to making the desired product **3.1** in acceptable yield (Scheme 3.8).

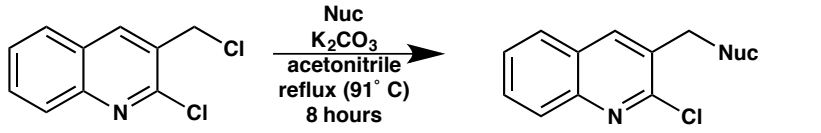


Scheme 3.8: Synthesis of **3.1** from the intermediate product **3.2**

Even according to the literature, the yield for the reaction is only 63%. At most we were only able to obtain a 55% yield, though it varied drastically from 11% to 55%. The reaction proved very susceptible to the conditions in the lab, which can explain the

varied yield. Furthermore, the solid that formed proved difficult to isolate. The literature called to wash the product with water and saturated sodium bicarbonate during filtration, but most times more product would precipitate out of the filtrate. The product needed to be filtered three or more times, and the isolated material was impure and required additional chromatography before use. The reaction was not run under argon; doing so could improve the yield. The usefulness of this reaction does not lie in the yield, but in its ability to be performed on large scales with cheap starting materials. Often times the scale used were ten or more grams, so even with a small yield, a large amount of product was obtained. This allowed for the use of this product to generate the **3.1** starting material in enough yield to use to generate a library of QMPs.

With the di-halogenated compound in hand, the general procedure of an S_N2 and a NAS reaction was followed to generate the library for this framework. The amines utilized for the library of compounds were pyrrolidine, 2-methylamine, piperidine, and diethylamine. These amines were shown to have the most promise on our current lead compound pyridine's framework in realkylating aged AChE in our kinetic studies. For initial testing, only the four were made to see if the framework has an activity (Scheme 3.9).



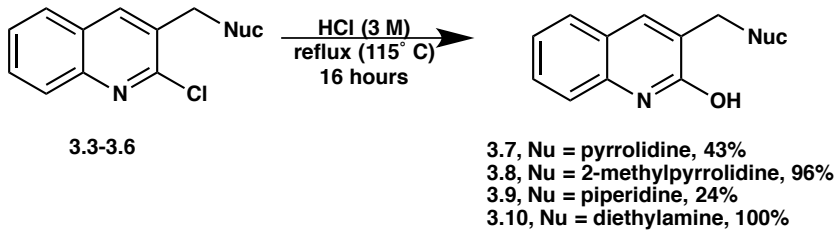
3.1

3.3, Nu = pyrrolidine, 98%
3.4, Nu = 2-methylpyrrolidine, 100%
3.5, Nu = piperidine, 96%
3.6, Nu = diethylamine, 100%

Scheme 3.9: S_N2 reaction of **3.1** and various amine nucleophiles

This framework allows for variability in the amines, as other amines and nucleophiles could be added to further build upon the library of this framework and hone in on their realkylating abilities. The yields of the S_N2 reactions were consistently extremely high at 96-100%.

Once the amine was placed onto the framework, an NAS reaction was performed to exchange the chlorine on the ring with a hydroxyl group (Scheme 3.10). The yields for this reaction varied greatly, from ~20-100%. It seems that some frameworks take longer than others to exchange the chlorine for the hydroxyl group under these conditions. For example, **3.9** proved nearly impossible to convert, and after 16 hours, only 24% of had converted and was isolatable. However, for **3.10**, the conversion under these conditions happened relatively fast and efficiently and after 16 hours full conversion was observed. This was the final step in making the desired quinone methide precursor that will go on to kinetic testing to see if it has the ability to realkylate aged AChE and restore activity in the enzyme. The kinetic results of compounds **3.7-3.10** will be discussed later on in this thesis.



3.3-3.6

3.7, Nu = pyrrolidine, 43%
3.8, Nu = 2-methylpyrrolidine, 96%
3.9, Nu = piperidine, 24%
3.10, Nu = diethylamine, 100%

Scheme 3.10: NAS reaction of **3.3-3.6** with 3 M hydrochloric acid to build final QMP compounds for testing, **3.7-3.10**

When analyzing the ^{13}CNMR data, the carbon type amount did not match what was expected. For **3.7**, there are expected to be 9 carbon types in the aromatic region

of 115 ppm to 165 ppm. However, there are 18 carbon types observed in the aromatic region of this compound, exactly twice the number as expected (Figure 3.3).

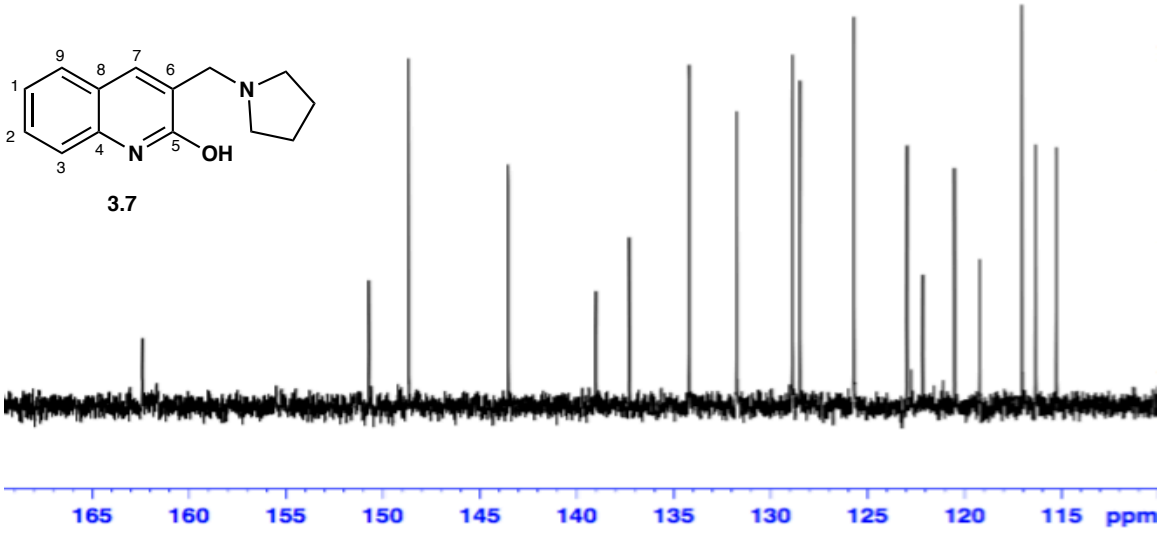


Figure 3.3: Zoomed in aromatic region of ^{13}CNMR for **3.7**, 18 signals were observed

To figure out what was causing the other carbon signals, a IR spectrum was taken of the compound. More peaks than expected were observed (Figure 3.4). The IR spectrum showed a broad peak designating the expected hydroxyl group around 3400 cm^{-1}. There is also a sharp peak around 1638 cm^{-1} designating a carbonyl. It seems that the tautomer of **3.7** is mixed in with the expected structure since are both present in the NMR spectra and in the IR spectra. The expected peak of the conjugated carboxylic amide in the tautomer is at 1655 cm^{-1}, but 1638 cm^{-1} is close to this value and due to the extremely high intensity of this peak, it can be ruled out as just the stretch of an aromatic double bond.

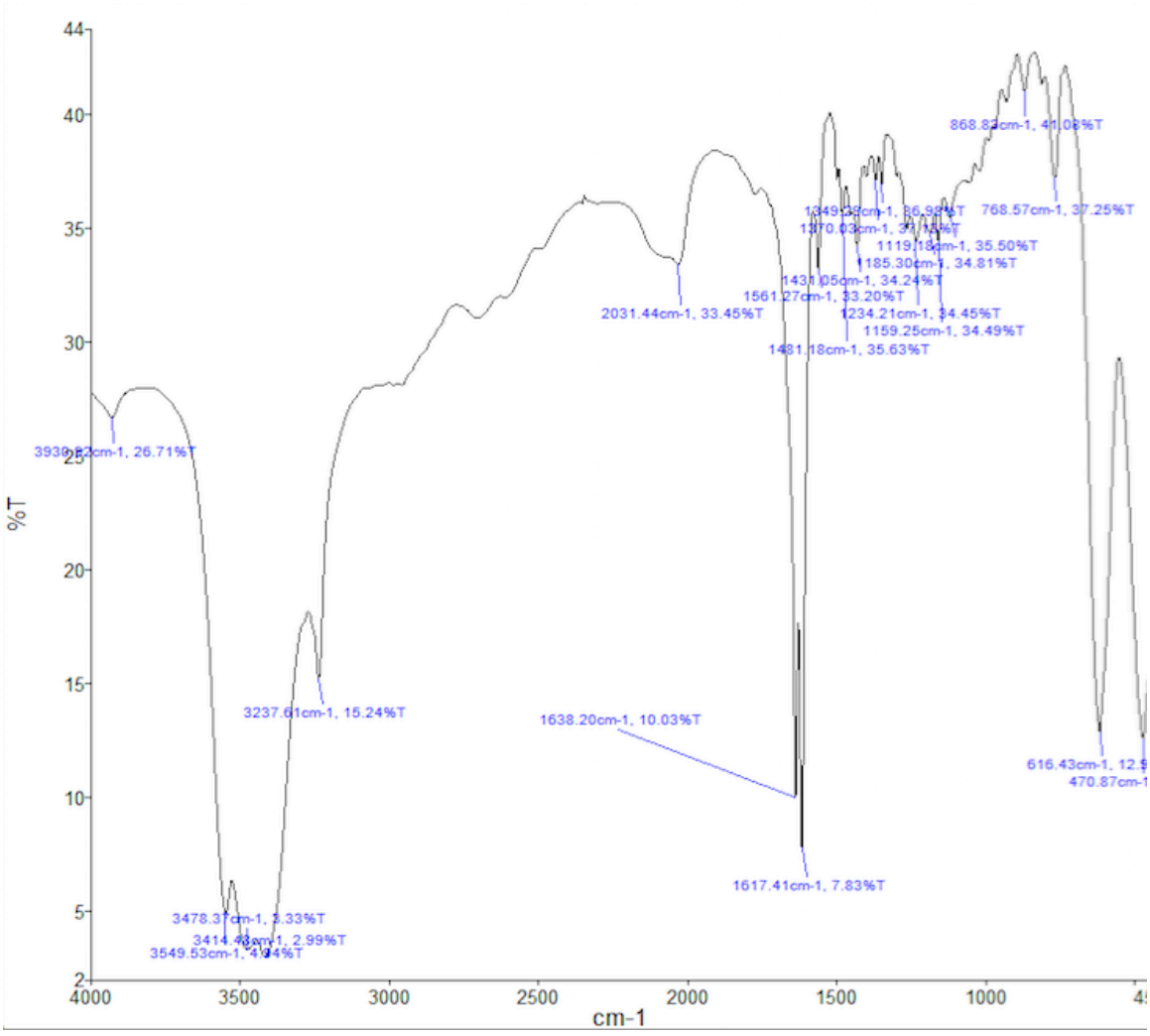


Figure 3.4: IR spectrum of **3.7**, features strong hydroxyl and carbonyl peaks

Since both forms of **3.7** have 9 carbons in the aromatic range, if both are present in the solid at the same time then it explains the 18 aromatic carbon types observed in the experimental ^{13}CNMR spectrum. Since both were not able to be separated, computational studies were sought to determine which is favored in solution and how this might affect their ability to perform as realkylators. For the current pyridine version with a hydroxyl on the two position, the tautomer was not observed in the spectrum, so

the equilibrium for the tautomers must be different for pyridine than quinoline. When computed using density functional theory at the M06-2X/6-31+G* level of theory and using the SMD solvation model (water), the ΔG_{298} values were -10.3 kcal/mol in the gas phase and -9.28 kcal/mol in the aqueous phase (Figure 3.5). The high negative Gibbs free energy for this equilibrium shows that the quinolinone tautomer is favored over the quinolinol by a significant amount.

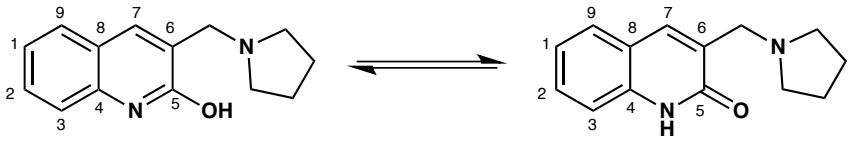
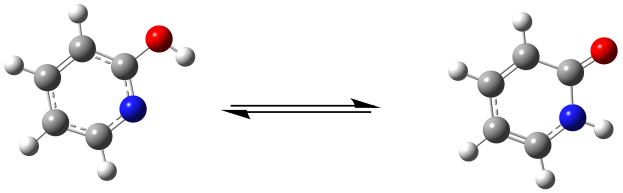


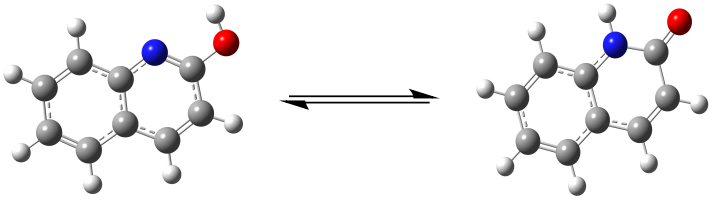
Figure 3.5: Tautomerization of **3.7**, favoring the quinolinone form over the quinolinol form

To see the difference between quinoline and pyridine in this respect, calculations were done on pyridinol and quinolinol tautomerizations to see where the equilibrium lies. When computed at the M06-2X/6-31+G* level and using the SMD solvation model (water), the ΔG_{298} values were 0.46 kcal/mol in the gas phase and -4.14 kcal/mol in the aqueous phase for the tautomerization of pyridinol to pyridinone. For the tautomerization of quinolinol to quinolinone, the ΔG_{298} values were -0.39 kcal/mol in the gas phase and -7.04 kcal/mol in the aqueous phase (Figure 3.6). The Gibbs free energy in the gas phase for the pyridine framework favors the pyridinol, whereas in the gas phase, the quinolinone is favored. This shows a varied difference between the structures of these two compounds and the expected forms they might have in the

body. The quinolinol is readily seen forming the quinolinone, so it could also be more favored that the pyridinol to form the carbonyl derivate in the body, and might be favored to form the QM for this same reason. Though in the aqueous phase, both seem to favor the amide tautomer, quinolinone has a larger negative value than pyridinone so it can be said that is the more stable of the two and might require less energy to form a QM once in the body.



Computed gas-phase ΔG_{298} = 0.46 kcal/mol
Computed with solvation (aq) ΔG_{298} = -4.14 kcal/mol



Computed gas-phase ΔG_{298} = -3.9 kcal/mol
Computed with solvation (aq) ΔG_{298} = -7.04 kcal/mol

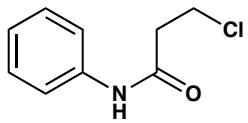
Figure 3.6: Computational tautomerization of pyridinol and quinolinol frameworks, respectively

The Vilsmeier-Haack reaction proves synthetically useful in generating frameworks to make substituted quinolines, and can be useful in the future to make other QMP frameworks. The products formed for testing as QMPs proved to be a mixture of tautomers of the hydroxyl and carbonyl forms, which could be interesting to keep into consideration when testing these compounds realkylation abilities on aged

AChE. The method discussed provided an inexpensive and efficient way to synthesize a library of 2-hydroxy-3-(aminomethyl)quinoline compounds.

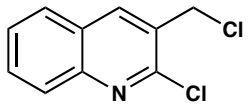
3.3 Experimental Data

General Methods The solvents used in these experiments were not purified any further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). Infrared spectra were recorded on a PerkinElmer Spectrum RX1 FTIR spectrometer. Absorptions are reported in wave numbers (cm^{-1}). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



3.2

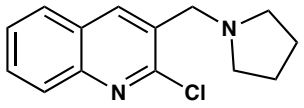
3-chloro-*N*-phenylpropanamide (3.2) To a solution of water (30 mL), sodium carbonate (3.76 g, 35.43 mmol), and aniline (5.87 mL, 64.43 mmol) was added 3-chloropropionyl chloride (6.77 mL, 70.87 mmol) dropwise over a 2 h period. The mixture was stirred for an additional 2 h and the resulting solids were collected via vacuum filtration, washing with hydrochloric acid (6M, 1 x 30 mL) and water (1 x 50 mL). The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.2** (11.033 g, 93%) as a white powdery solid: R_f 0.69 (10:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.58–7.48 (m, 2 H), 7.37–7.29 (m, 2 H), 7.17–7.11 (m, 1 H), 3.89 (t, 2 H, J = 6.4), 2.82 (t, 2 H, J = 6.4), 1.56 (br s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 167.60, 137.40, 129.08, 124.72, 120.02, 40.61, 39.87; HRMS (ESI) calcd for (M+Na) $C_9H_{10}ClNO$: 206.0338, found 206.0348.



3.1

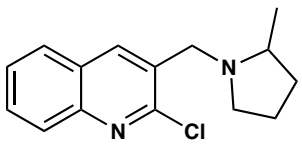
2-chloro-3-(chloromethyl)quinoline (3.1) To a solution of dimethylformamide (9.30 mL, 120.15 mmol) at 0 ˚C was added phosphorus oxychloride (39.20 mL, 420.54 mmol) dropwise over 1.5 h. The reaction mixture was allowed to warm to ambient temperature over 30 min followed by the addition of **3.2** (11.03 g, 60.08) over 5 min. The mixture was stirred for 20 min and then heated to 85 °C for 4 h. The reaction was cooled to rt and then poured over crushed ice (100 g). The solids which precipitated were collected via vacuum filtration and washed with water (2 x 100 mL), 10% $NaHCO_3$ (1 x 100 mL), and water (1 x 100 mL). The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.1** (5.1629 g, 100%) as a white powdery solid: R_f 0.41 (10:1,

hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.31 (d, 1 H, *J* = 1.2 Hz), 8.03 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.85 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.76 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.60 (td, 1 H, *J* = 1.2, 8.0 Hz), 4.85 (s, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 149.70, 147.37, 138.75, 131.01, 129.12, 128.37, 127.62, 127.52, 127.14, 43.15; HRMS (ESI) calcd for (M+H) $C_{10}H_7Cl_2N$: 212.0028, found 212.0019.



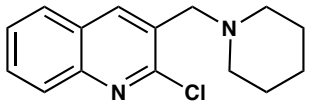
3.3

2-chloro-3-(pyrrolidin-1-ylmethyl)quinoline (3.3) To a solution of acetonitrile (10 mL), **3.1** (2.50 g, 1.18 mmol), and potassium carbonate (0.163, 1.18 mmol) was added pyrrolidine (0.20 mL, 2.36 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **3.3** (0.285 g, 98%) as a yellow oil: R_f 0.22 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.80 (d, 1 H, *J* = 1.2 Hz), 8.09 (dd, 1 H, *J* = 1.2, 8.4 Hz), 8.00 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.90 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.60 (td, 1 H, *J* = 1.2, 8.0 Hz), 4.94 (s, 2 H), 2.30–2.22 (m, 4 H), 2.28–2.15 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.71, 148.66, 137.28, 134.19, 128.86, 125.69, 120.53, 117.05, 116.34, 52.45, 45.21, 22.50; HRMS (ESI) calcd for (M+H) $C_{14}H_{15}ClN_2$: 247.7455, found 247.1001.



3.4

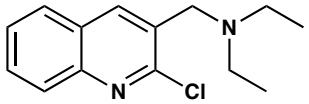
2-chloro-3-((2-methylpyrrolidin-1-yl)methyl)quinoline (3.4) To a solution of acetonitrile (10 mL), **3.1** (0.250, 1.18 mmol), and potassium carbonate (0.163 g, 1.18 mmol) was added pyrrolidine (0.24 mL, 2.36 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **3.4** (0.314 g, 100%) as a yellow powdery solid: R_f 0.85 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 8.27 (d, 1 H, *J* = 1.2 Hz), 8.00 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.82 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.69 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.54 (td, 1 H, *J* = 1.2, 8.0 Hz), 4.15 (d, 1 H, *J* = 15.2) 3.52 (d, 1 H, *J* = 15.2), 3.23–3.15 (m, 1 H), 2.68–2.56 (m, 1 H), 2.22 (q, 1 H, *J* = 8.8), 2.09–1.97 (m, 1 H), 1.89–1.68 (m, 2 H), 1.56–1.44 (m, 1 H), 1.20 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.88, 146.69, 137.99, 132.07, 129.77, 128.21, 127.56, 127.39, 126.84, 60.17, 55.04, 54.50, 32.80, 21.96, 19.38; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1080, found 261.1465.



3.5

2-chloro-3-(piperidine1-ylmethyl)quinoline (3.5) To a solution of acetonitrile (10 mL), **3.1** (0.250 g, 1.18 mmol), and potassium carbonate (0.163 g, 1.18 mmol) was added pyrrolidine (0.23 mL, 2.36 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer

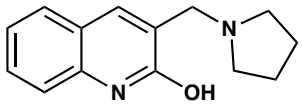
was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **3.5** (0.295 g, 96%) as a white powdery solid: R_f 0.55 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 8.26 (d, 1 H, *J* = 1.2 Hz), 7.99 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.82 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.69 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.54 (td, 1 H, *J* = 1.2, 8.0 Hz), 3.69 (s, 2 H), 2.58–2.46 (m, 4 H), 1.78–1.59 (m, 4 H), 1.54–1.40 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.15, 146.70, 137.88, 130.94, 129.80, 128.20, 127.49, 127.44, 126.85, 59.77, 54.84, 26.11, 24.29; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1153, found 261.1466.



3.6

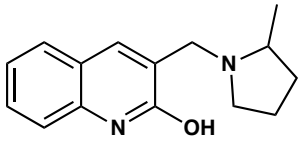
***N*-((2-chloro-quinoline-3-yl)methyl)-*N*-ethylethanamine (3.6)** To a solution of acetonitrile (10 mL), **3.1** (0.250, 1.18 mmol), and potassium carbonate (0.163 g, 1.18 mmol) was added pyrrolidine (0.24 mL, 2.36 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **3.6** (0.293 g, 100%) as a white powdery solid: R_f 0.55 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 8.31 (d, 1 H, *J* = 1.2 Hz), 8.00 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.82 (dd, 1 H, *J* = 1.2, 7.2 Hz), 7.68 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.54 (td, 1 H, *J* = 1.2, 8.0 Hz), 3.78 (s, 2 H), 2.65 (q, 4 H, *J* = 7.2 Hz), 1.10 (t, 6 H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C)

150.88, 146.66, 137.80, 132.46, 129.70, 128.18, 127.61, 127.43, 126.82, 54.59, 47.56, 12.11; HRMS (ESI) calcd for (M+H) $C_{14}H_{17}ClN_2$: 249.1080, found 249.1447.



3.7

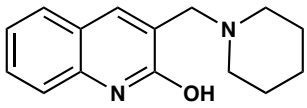
3-((2-methylpyrrolidin-1-yl)methyl)quinolin-2-ol (3.7) To a solution of hydrochloric acid (3 M, 5 mL) was added **3.3** (0.150 g, 0.608 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.7** (0.172 g, 74%) as a white powdery solid: R_f 0.13 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 8.23 (d, 1 H, *J* = 1.2 Hz), 7.78 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.77–7.56 (m, 2 H), 7.33 (td, 1 H, *J* = 1.6, 8.4 Hz), 3.37 (s, 2 H), 3.48–3.21 (m, 4 H), 2.20–2.01 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 150.72, 143.52, 137.28, 131.74, 128.48, 122.95, 123.64, 119.23, 116.34, 55.58, 52.46, 22.51; HRMS (ESI) calcd for (M+H) $C_{14}H_{16}N_2O$: 229.1335, found ???.



3.8

3-((2-methylpyrrolidin-1-yl)methyl)quinolin-2-ol (3.8) To a solution of hydrochloric acid (3 M, 5 mL) was added **3.4** (0.150, 0.608 mmol). The reaction mixture was refluxed

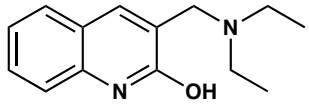
for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.8** (0.134 g, 96%) as an off-white powdery solid: R_f 0.13 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 7.91 (d, 1 H, *J* = 1.2 Hz), 7.58 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.46 (dd, 1 H, *J* = 1.2, 6.8 Hz), 7.36 (td, 1 H, *J* = 1.6, 8.4 Hz), 7.18 (td, 1 H, *J* = 1.6, 8.4 Hz), 3.99 (d, 1 H, *J* = 16.0) 3.45 (d, 1 H, *J* = 16.0), 3.20–3.11 (m, 1 H), 2.66–2.54 (m, 1 H), 2.22 (q, 1 H, *J* = 8.8), 2.06–1.94 (m, 1 H), 1.90–1.62 (m, 2 H), 1.58–1.43 (m, 1 H), 1.21 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 163.87, 137.56, 137.44, 131.50, 129.57, 127.53, 122.42, 120.27, 115.49, 60.06, 54.47, 51.93, 32.77, 21.96, 19.41; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1495.



3.9

3-(piperidin-1-yl)methyl)quinolin-2-ol (3.9) To a solution of hydrochloric acid (3 M, 5 mL) was added **3.1** (0.150 g, 0.575 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.9** (0.033 g, 24%) as a yellow oil: R_f 0.38 (1:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 7.92 (d, 1 H, *J* = 1.2 Hz), 7.71 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.60 (dd, 1 H, *J* = 1.2, 6.8 Hz), 7.51 (td, 1 H, *J* = 1.6, 8.4 Hz), 7.20

(td, 1 H, *J* = 1.6, 8.4 Hz), 3.79 (s, 2 H), 2.60–2.47 (m, 4 H), 2.00–1.89 (m, 4 H), 1.82–1.71 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 157.47, 146.88, 138.02, 128.79, 126.90, 126.12, 123.64, 123.14, 121.85, 58.41, 54.02, 49.66, 29.72, 25.74, 23.58; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1497.



3.10

3-((diethylamino)methyl)quinolin-2-ol (3.10) To a solution of hydrochloric acid (3 M, 5 mL) was added **3.1** (0.150 g, 0.603 mmol). The reaction mixture was refluxed for 16 h. The reaction was diluted with water, neutralized with potassium carbonate, and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **3.10** (0.168 g, 110%) as a white powdery solid: R_f 0.13 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)) 7.96 (d, 1 H, *J* = 1.2 Hz), 7.58 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.45 (dd, 1 H, *J* = 1.2, 6.8 Hz), 7.33 (td, 1 H, *J* = 1.6, 8.4 Hz), 7.20 (td, 1 H, *J* = 1.6, 8.4 Hz), 3.66 (s, 2 H), 2.65 (q, 4 H, *J* = 7.2), 1.11 (t, 6 H, *J* = 7.2); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 163.86, 137.27, 137.14, 131.98, 129.50, 127.56, 122.43, 120.35, 115.40, 51.59, 47.72, 12.21; HRMS (ESI) calcd for (M+H) $C_{14}H_{18}N_2O$: 231.1492, found 231.0496.

3.4 References for Chapter 3

(1) Calvin, J. R.; Hillstrom, G. F.; Holland, J.; Krieger, P. E.; Murugan, R.; Scriven, E. F. V.; J. Yang. *ARKIVOC*. **2002**, *6*, 257-263.

(2) Vilsmeier, A.; Haack, A. *Ber. dtsch. Chem. Ges.* **1927**, *60*, 119–122.

CHAPTER FOUR

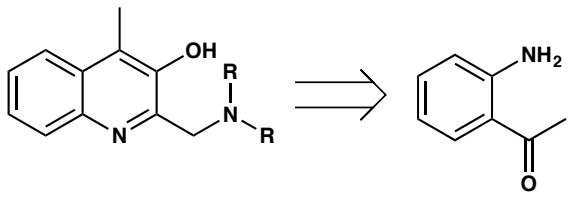
SYNTHESIS OF 3-HYDROXY-2-(AMINOMETHYL)-4-METHYLQUINOLINE

4.1 Introduction

Computationally, the framework that has shown the most promise in the quinolines is based on the lead pyridine framework. As previously discussed, the lead pyridine framework is based of 3-hydroxy-2-(aminomethyl)pyridine. One of the main substituted quinoline frameworks to investigate would be 3-hydroxy-2-(aminomethyl)quinoline, to compare how adding the second ring system affects the ability of this compound to resurrect aged AChE.

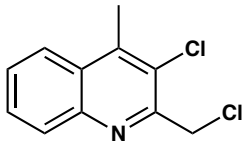
Unfortunately, finding starting material to generate this quinoline framework has proven very difficult. There are few commercially available quinolines with a modifiable functional group such as chlorine or hydroxyl groups placed in the three position of the quinoline ring. In order to hone in the framework's ability to realkylate, conditions were sought synthesize similar quinoline structures that at least could have the hydroxyl in the three position and the leaving group in the two position. It is found in the literature that small amine-containing compounds of reasonable price can be used to synthesize quinolines. As previously discussed, aniline and acyl chlorides have been used to generate quinolines as they already contain an amine and one of the benzene rings. We hypothesized that other aniline derivatives could be used to obtain our desired framework. As 2-aminoacetophenone is available and inexpensive compared to other substituted anilines, a version of 3-hydroxy-2-(aminomethyl)quinoline was sought after with a methyl group *ortho* to the hydroxyl to see if the leaving group and hydroxyl group positions showed activity, regardless of the addition of the electron donating methyl on

the ring (Scheme 4.1). Our goal is to find an inexpensive way to synthesize the di-halogenated compound. Once this compound is made with two halogens, a S_N2 and NAS could be performed to make the desired QMP libraries, like in Scheme 2.2.



Scheme 4.1: Retrosynthesis of 2-hydroxy-3-(aminomethyl)-4-methylquinoline from 2-aminoacetophenone

By having the ability to perform an S_N2 and vary the leaving group, this enables the variation of the nucleophiles that can be placed onto the framework and tested for their ability to resurrect aged AChE (Figure 4.1). The ability to vary the leaving group that will go on to generate the QM is vital to allow us to screen a variety of compounds and be able to hone in on what makes a compound a significant realkylator of the enzyme.



4.1

Figure 4.1: Desired dihalogenated quinoline to generate a QMP library from

Also, by being able to perform the NAS reaction last, it simplifies the isolation and purifications of these compounds since they are easier to clean up and extract when they are less polar.

This chapter will discuss the synthesis of a library of QMPs based on the general framework of 2-hydroxy-3-(aminomethyl)-4-methylquinoline.

4.2 Results, Discussion, and Conclusions

2-Aminoacetophenone is commonly used in the literature to generate substituted quinoline frameworks (Figure 4.2). Common syntheses for quinoline with 2-aminoacetophenone involve the use of tin, copper, and a variety of other metals to catalyze the conversion. The literature does contain some successful reactions with these catalysts, but most in the literature involve many strenuous intermediate steps just to generate the substituted quinoline. Therefore, other reactions conditions were sought to be able to generate the quinoline in cost effective and time effective manner.

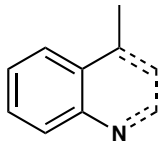
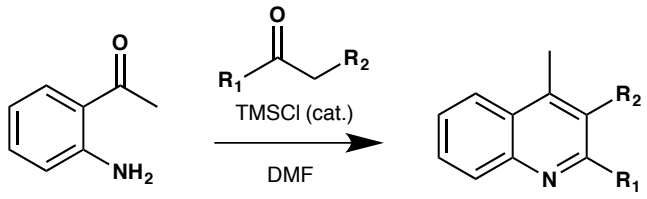


Figure 4.2: Use of 2-aminoacetophenone to generate quinoline framework

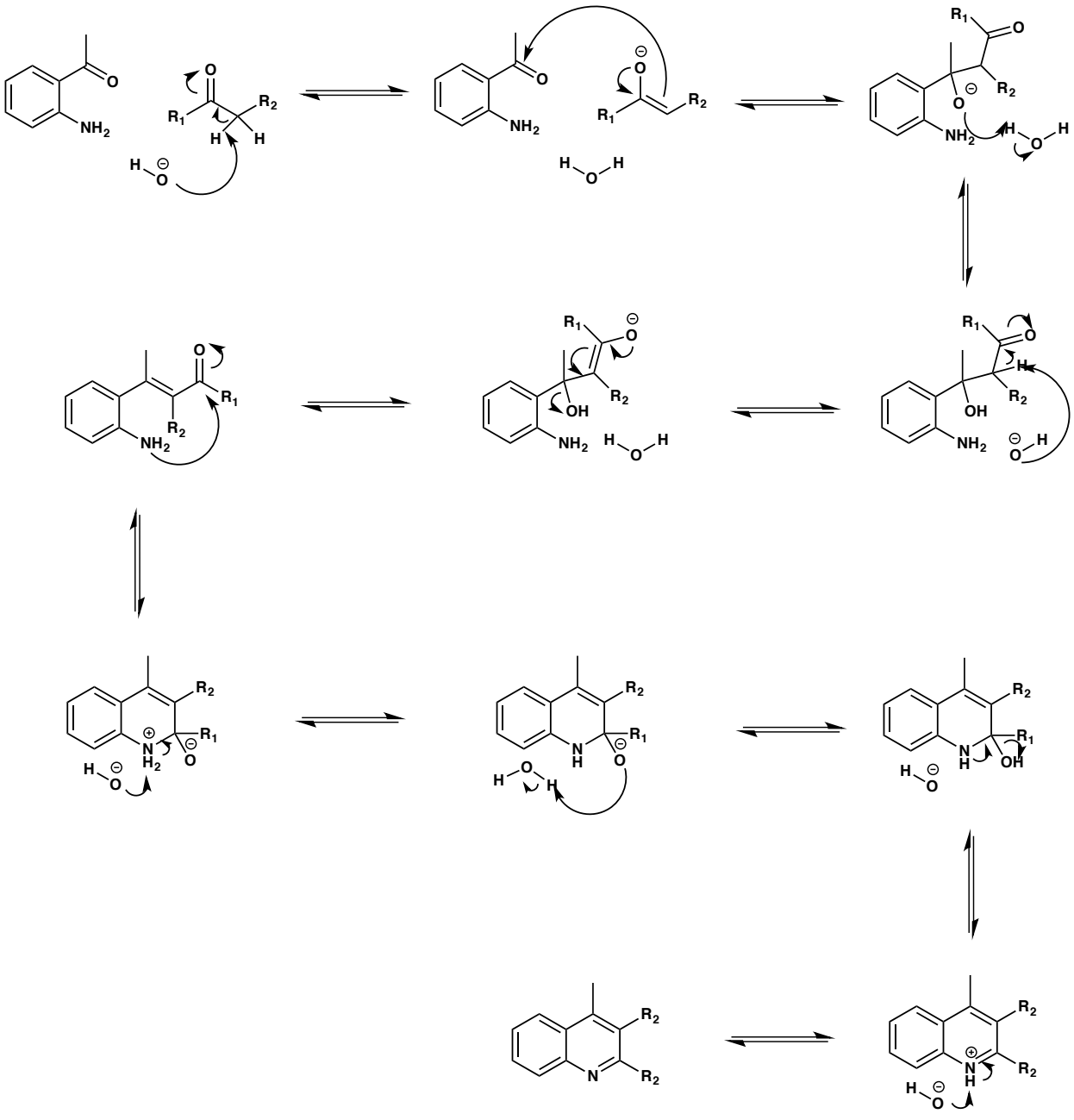
A synthesis was investigated from the literature that began with 2-aminoacetophenone and was used to make framework **4.1** without the use of heavy metals, but instead acid catalysts[1]. The reaction cited is known as the Friedlaender Annulation reaction and is a useful synthesis to develop substituted quinoline frameworks. The starting material for these reactions requires an *ortho* amino aryl carbonyl in order to ensure the carbonyl carbon and the nitrogen atoms are in the

correct placement to form a quinoline ring. The reaction also requires the use of a ketone with an α-methylene group[2]. After the initial condensation, the intermediate undergoes either an acid- or base-catalyzed cyclocondensation to produce the quinoline product. The common mechanism for a base-catalyzed Friedlaender synthesis is shown in Scheme 4.2. This reaction is known to cyclize these compounds when both the reagent and starting material contain ketone functional groups. The use of a functional group other than a ketone on the aniline will be discussed further in a future chapter.

The cited source uses trimethylsilyl chloride (TMSCl) as a catalyst[1]. The TMSCl speeds up the reaction compared to using common catalysts for these types of reactions, like sodium hydroxide. Using 2-aminoacetophenone as one of the starting materials, the other reagent ketone would need to be purchased or made with the proper substituents to ensure proper placement on the ring. According to scheme 4.3, the R groups can be variable and by altering them, one can edit the quinoline formed.



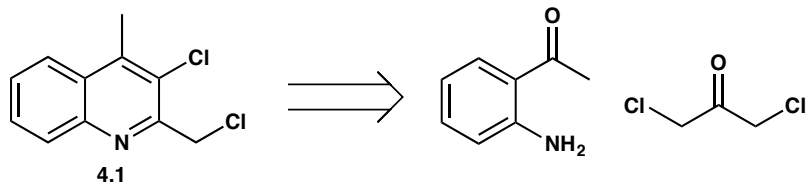
Scheme 4.3: Proposed reaction to generate the quinoline framework from 2-aminoacetophenone



Scheme 4.2: General base-catalyzed mechanism for the cyclocondensation of 2-aminoacetophenone and a ketone to form quinoline

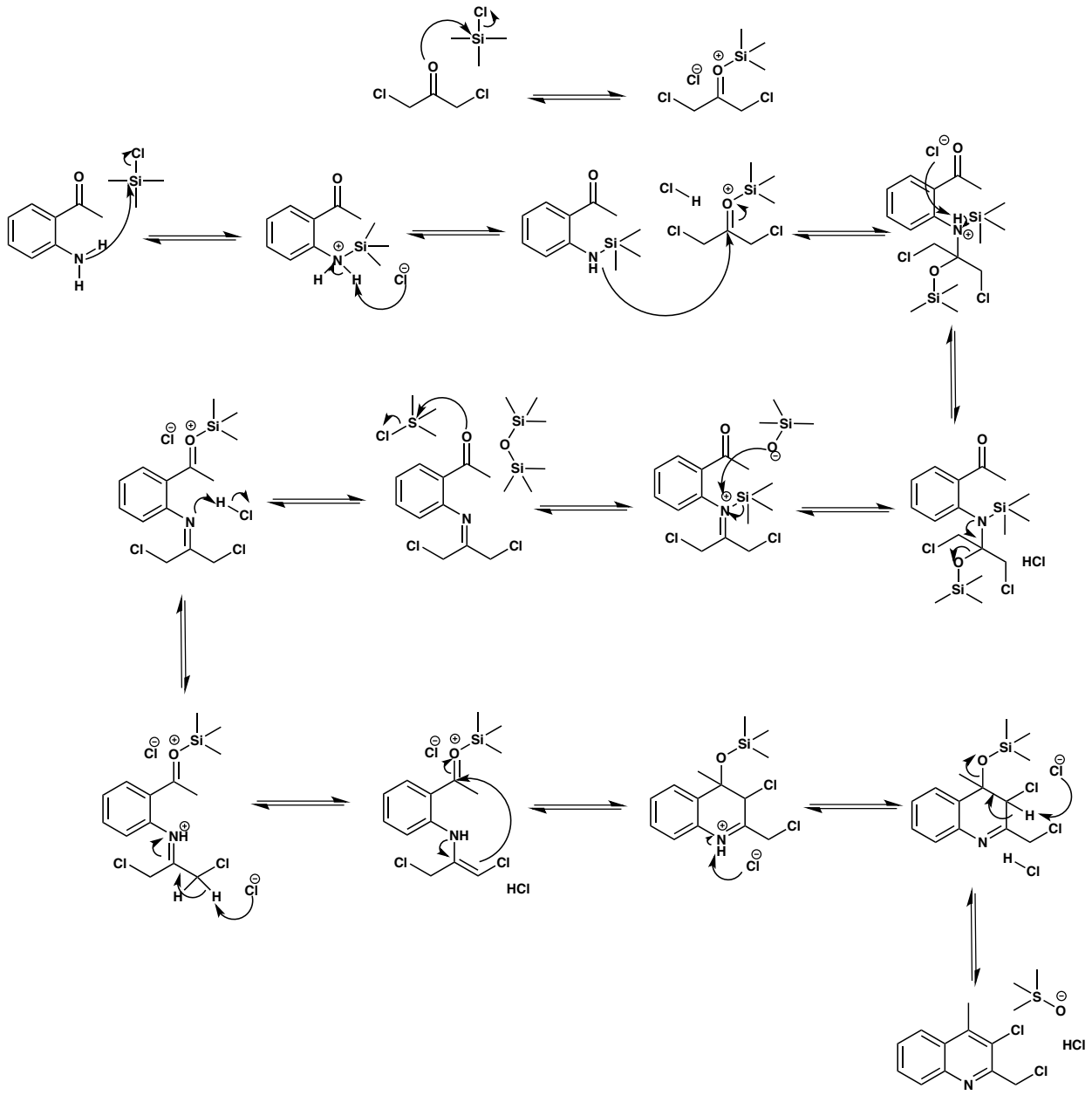
If the R_1 and R_2 groups are not identical, it could lead to mixed products as long as there is an α-hydrogen on both sides of the carbonyl. In order to avoid having to worry about side products, a symmetrical ketone would need to be used as the reagent.

A retrosynthesis was performed to determine that 1,3-dichloroacetone was the reagent to generate **4.1** (Scheme 4.4).



Scheme 4.4: Retrosynthetic analysis of the formation of **4.1** from 2-aminoacetophenone and 1,3-dichloroacetone

Under the TMSCl catalyzed conditions, the mechanism is different. Under the common base conditions, the reaction takes multiple hours to complete. However, for the TMSCl conditions, the reaction takes less than an hour. This makes this reaction even more useful as it can save time in making substrates for a large library of QMPs. The mechanism is shown in Scheme 4.5. The mechanism involves the coordination of the TMS group to the ketone in 1,3-dichloroacetone, followed by a nucleophilic substitution of the oxygen on the carbonyl for the chlorine on the silane, which causes the carbonyl carbon to become more electrophilic due to the resonance with the positively charged oxygen. A similar step happens with the amine in 2-aminoacetophenone as it nucleophilically attacks the silane group, which causes the nitrogen to become a better nucleophile. The nitrogen can then easily attack the electrophilic carbon of 1,3-dichloroacetone and eventually form an imine intermediate. The ketone on 2-aminoacetophenone also coordinates to TMSCl, making the carbonyl carbon a sufficient electrophile.

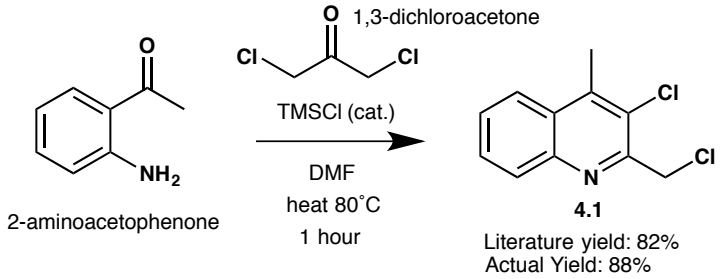


Scheme 4.5: Proposed mechanism for the formation of **4.1** from 2-aminoacetophenone and 1,3-dichloroacetone under TMSCl catalyzed conditions[3]

After a tautomerization of the imine to an enamine, electrons from the double bond can attack the electron deficient carbon of the ketone to cyclize the molecule. With a subsequent dehydration with the removal of the –OTMS group, the final quinoline

product **4.1** is formed. As seen in the mechanism, the R group should be able to be varied. It would also be nearly impossible to use an asymmetrical ketone instead of 1,3-dichloroacetone since it would show no preference for one product over the other as long as both sides of the ketone have α-hydrogens.

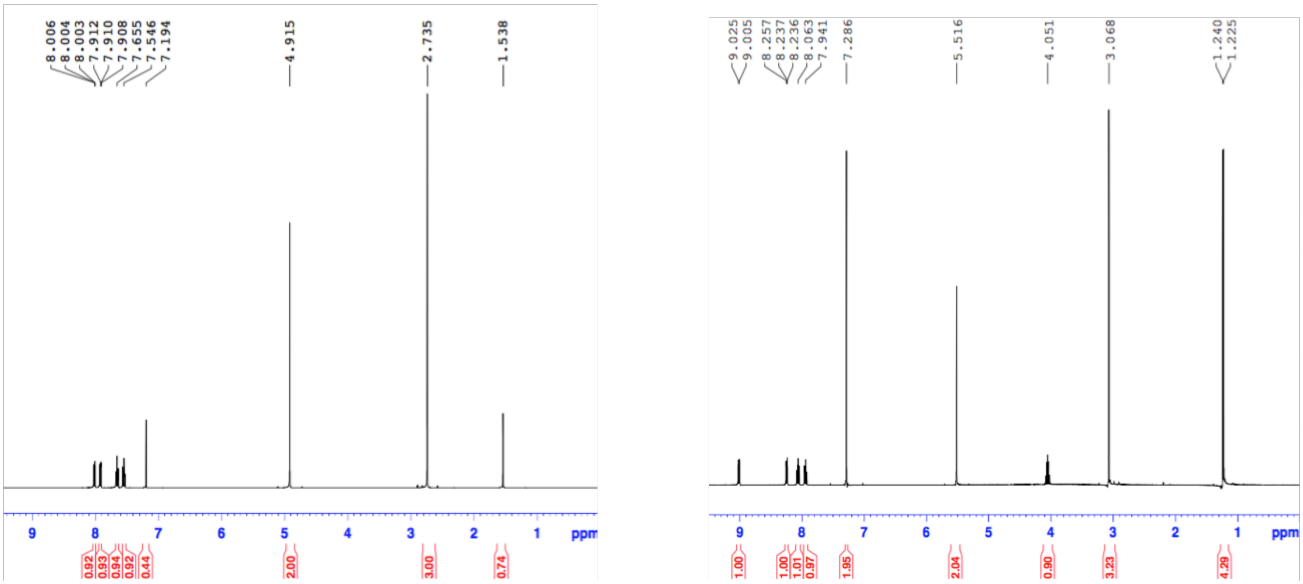
Having water in the system could potentially ruin the reaction as it itself could start coordinating to the silane groups and also could ruin the imine formed in solution. Therefore, the reaction is run in DMF under anhydrous conditions to ensure high yields of the reaction. The mixture of both 1,3-dichloroacetone and 2-aminoacetophenone proved to be a suitable choice to making the desired product **4.1** in high yield, even higher than obtained in the literature (Scheme 4.6).



Scheme 4.6: Synthesis of **4.1** from 2-aminoacetophenone and 1,3-dichloroacetone

There are problems with this TMSCl catalyzed reaction when performed on large scales. The work-up for this reaction involves quenching with water, subsequently cooling in an ice bath, and then isolating via vacuum filtration, washing with isopropyl alcohol. When performed on a 0.250 g scale of 2-aminoacetophenone, the solid crystallized out and was able to be filtered easily as the pure product. By increasing the

scale to 3.00 g, however, impure product precipitates from the reaction. The solid was no longer crystalline. An ^{1}HNMR analysis was conducted on the two products, one being from the 0.250 g scale and one from the 3.00 g scale. It seems that even the aromatic hydrogens from the large-scale product were deshielded and shifted downfield (Figure 4.3). The literature melting point is 101-102 ˚C, but the melting point of the product obtained was 200-212 ˚C.



Small Scale

Large Scale

Figure 4.3: ^{1}HNMR spectra for the 0.250 g scale and 3.00 g scale, respectively

The mass of the product was also obtained. It showed none of the expected ratios for chlorine isotopes. The mass also differed significantly from the expected value, and did not match the [M+H], [M+Na], or [M+K] peaks (Figure 4.4). The reaction

was repeated and attempted to be extracted from dichloromethane, but the solid that was obtained from the organic layer also contained the same impurities.

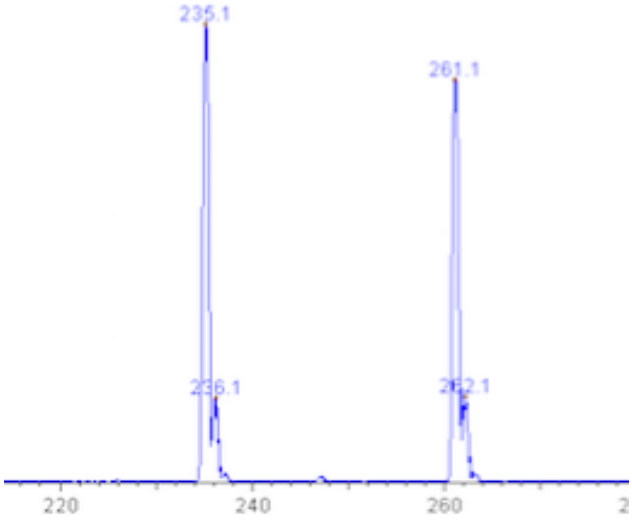
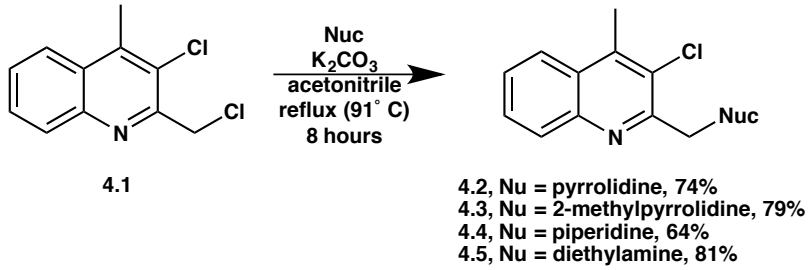


Figure 4.4: Zoomed in mass spectra for the 3.00 g scale; Expected [M+H] 226, [M+Na] 248, [M+K] 264; expected chlorine ratios 100%: 63.9%: 10.2%; Observed 235, 261

It seems that this reaction is therefore scale dependent. In order to see if a high yield could be obtained on a larger scale than 0.250 g, the reaction was repeated on both 1.00 g and 2.00 g scales. While the 2.00 g scale also had a low yield, the 1.00 g allowed for product formation in high yield that was easily separated from the few insoluble salts using column chromatography. The 1.00 g scales afforded sufficient yield to generate enough of **4.1** to complete the library of QMPs.

The general procedure of an S_N2 was performed on **4.1** to generate the library for this framework. The amines utilized for the library of compounds were pyrrolidine, 2-methylpyrrolidine, piperidine, and diethylamine. These amines were shown to have the most promise on our current lead compounds in realkylating aged AChE. The kinetic

studies on them proved that they were superior to other amines places on the ring system. For initial testing, only the four were made to see if the framework has any activity (Scheme 4.7).



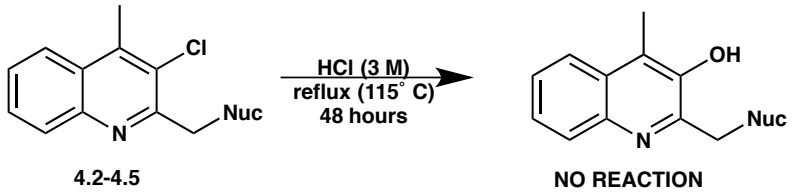
4.1

4.2, Nu = pyrrolidine, 74%
4.3, Nu = 2-methylpyrrolidine, 79%
4.4, Nu = piperidine, 64%
4.5, Nu = diethylamine, 81%

Scheme 4.7: S_N2 reaction of **4.1** and various amine nucleophiles

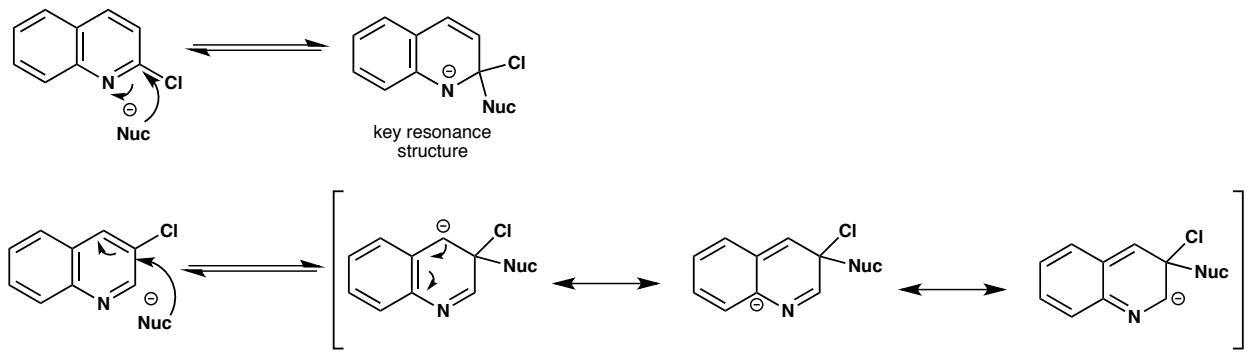
This framework allows for variability with the amines and nucleophiles, as many varied nucleophiles can be placed onto the ring at position two. This could go on to further expand the library of these QMPs to hone in on their realkylating abilities for AChE. The yields of the S_N2 reactions were a bit lower than seen for the previous frameworks, with a range of around 60-80%. This was still high enough to generate enough desired product to use in QMP synthesis.

The NAS reaction used in previous frameworks was attempted on these frameworks to exchange the chlorine on the ring with a hydroxyl group. However, standard reaction would not work for this framework, even over a period of 48 hours (Scheme 4.8).



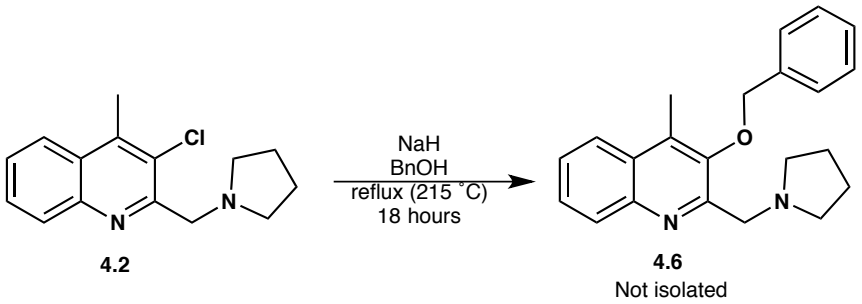
Scheme 4.8: Attempted NAS reaction of **4.2-4.5** with 3 M hydrochloric acid

In order to generate the QMP framework, a hydroxyl group is needed. The other frameworks previously discussed that had successful NAS reactions had the chlorine on the 2-position rather than the 3-position. At the 2-position, the intermediate formed in the NAS reaction is able to disperse the negative charge formed onto the nitrogen atom. This resonance form helps to stabilize this intermediate. However, at the 3-position, the intermediate formed is not is not able to disperse the charge onto the nitrogen atom, so the charge is only dispersed on the carbon atoms, which is not favored (Scheme 4.9).



Scheme 4.9: First step in NAS reaction, the 2-position forms key intermediate with charge on nitrogen where as the 3-position is not able to stabilize the intermediate

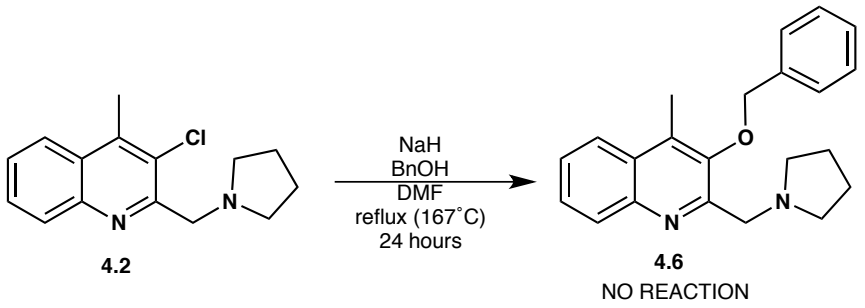
Since these conditions did not work to generate the QMP framework, other reaction conditions were sought out for this conversion. If the chlorine could instead be converted to a oxygen with a benzyl group on it, then later the benzyl group could easily be removed later with palladium on carbon in a hydrogen atmosphere to leave a free hydroxyl. By using benzyl alcohol as the solvent rather than water for this NAS reaction, the temperature is able to increase almost 100 ˚C (Scheme 4.10). The TLC showed product formation within two hours and full conversion after 16 hours.



4.2 **4.6** Not isolated

Scheme 4.10: Attempted synthesis of **4.6** from **4.2** using benzyl alcohol

Though product was observed by TLC, it was not able to be separated out from the solution. The benzyl alcohol proved extremely difficult to separate out. The mixture was extracted with acid and then with base to see if they could separate, but both were present in both layers. The reaction was attempted in DMF instead but no conversion was observed (Scheme 4.11). At 215 ˚C, product formation was observed within 2 hours. Since the reaction mixture was difficult to separate from benzyl alcohol, other reaction conditions are needed to optimize this conversion and isolate the desired product.



Scheme 4.11: Attempted synthesis of **4.6** from **4.2** using DMF as the solvent

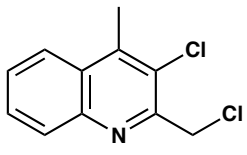
Currently, no reactions have been successful in converting **4.2-4.5** to the desired QMP structure. We are investigating the use of boron to help convert the chlorine to a hydroxyl group to generate the QMP library. Though not QMPs, the ability of **4.2-4.5** to resurrect aged AChE will be kinetically tested to see how they compare to the hydroxyl frameworks and to see if even though they cannot form a QMP, they can still realkylate through an alternate mechanism.

The Friedlaender Annulation reaction proves synthetically useful to generate substituted quinoline frameworks in high yields. The reaction can be useful to make other QMP frameworks, one of which will be discussed in a future chapters. The method discussed provided an inexpensive and efficient way to synthesize the 3-chloro-2-(aminomethyl)-4-methylquinoline framework that can still generate a library of compounds, though not yet the desired QMP compounds until conditions to convert the chlorine to a hydroxyl group are discovered.

4.3 Experimental Data

General Methods The solvents used in these experiments were not purified any

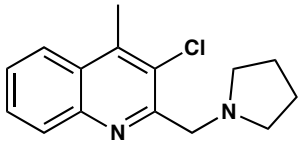
further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



4.1

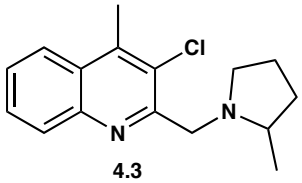
3-chloro-2-(chloromethyl)-4-methyl-quinoline (4.1) To a solution dimethylformamide (5 mL), 2-aminoacetophenone (1.00 g, 7.40 mmol), 1,3-dichloroacetone (0.939 g, 7.40 mmol) was added TMSCl (3.56 mL, 27.97 mmol). The reaction mixture was heated to 80 °C for 1 h. The reaction was cooled to rt and then diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (10:1 hexanes:EtOAc) to yield **4.1** (1.49 g, 88%) as a white powdery solid: R_f 0.50 (10:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.08 (dd, 1 H, *J* =

1.2, 7.6 Hz), 7.99 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.72 (td, 1 H, *J* = 1.2, 7.2 Hz), 7.60 (td, 1 H, *J* = 1.2, 7.2 Hz), 4.98 (s, 2 H), 2.81 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 153.31, 145.41, 142.70, 130.16, 129.54, 128.33, 127.97, 127.78, 123.83, 46.11, 15.61; HRMS (ESI) calcd for (M+Na) $C_{11}H_9Cl_2N$: 248.0004, found 247.9993.



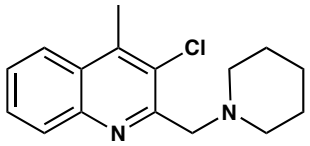
4.2

3-chloro-4-methyl-2-(pyrrolidin-1-ylmethyl)quinoline (4.2) To a solution of acetonitrile (10 mL), **4.1** (1.279 g, 5.56 mmol), and potassium carbonate (0.782 g, 5.56 mmol) was added pyrrolidine (0.94 mL, 11.31 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **4.2** (1.088 g, 74%) as an light orange powdery solid: R_f 0.36 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.12 (dd, 1 H, *J* = 1.0, 7.6 Hz), 7.97 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.69 (td, 1 H, *J* = 1.2, 7.2 Hz), 7.57 (td, 1 H, *J* = 1.2, 7.2 Hz), 4.11 (s, 2 H), 2.84–2.71 (m, 7 H), 2.88-2.81 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 155.72, 145.39, 141.12, 130.27, 128.79, 128.54, 127.67, 126.67, 123.65, 60.39, 54.55, 23.61, 15.47; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1153, found 261.1142.



4.3

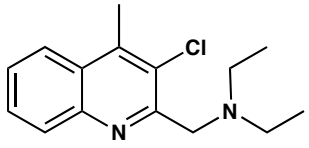
3-chloro-4-methyl-3-((2-methylpyrrolidin-1-yl)methyl)quinoline (4.3) To a solution of acetonitrile (10 mL), **4.1** (1.319, 5.83 mmol), and potassium carbonate (0.806 g, 5.83 mmol) was added pyrrolidine (1.19 mL, 11.67 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **4.3** (1.265 g, 79%) as a light orange powdery solid: R_f 0.40 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.10 (dd, 1 H, *J* = 1.0, 7.6 Hz), 7.95 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.68 (td, 1 H, *J* = 1.2, 7.2 Hz), 7.55 (td, 1 H, *J* = 1.2, 7.2 Hz), 4.52 (d, 1 H, *J* = 13.2) 3.58 (d, 1 H, *J* = 13.2), 3.22–3.15 (m, 1 H), 2.77 (s, 3 H), 2.68–2.56 (m, 1 H), 2.39 (q, 1 H, *J* = 9.2), 2.03–1.91 (m, 1 H), 1.83–1.65 (m, 2 H), 1.55–1.42 (m, 1 H), 1.25 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 156.29, 145.28, 141.30, 130.14, 128.95, 128.78, 127.77, 126.68, 123.69, 60.57, 58.85, 54.52, 32.64, 21.81, 19.14, 15.54; HRMS (ESI) calcd for (M+H) $C_{16}H_{19}ClN_2$: 275.1310, found 275.1296.



4.4

3-chloro-4-methyl-2-(piperidine1-ylmethyl)quinoline (4.4) To a solution of acetonitrile (10 mL), **4.1** (1.492 g, 6.60 mmol), and potassium carbonate (0.912 g, 6.60 mmol) was added pyrrolidine (1.30 mL, 13.20 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude

product was purified by chromatography (2:1 hexanes:EtOAc) to yield **4.4** (1.165 g, 64%) as a white oil: R_f 0.47 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.10 (dd, 1 H, *J* = 1.0, 7.6 Hz), 7.95 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.66 (td, 1 H, *J* = 1.2, 7.2 Hz), 7.54 (td, 1 H, *J* = 1.2, 7.2 Hz), 3.89 (s, 2 H), 2.77 (s, 3 H), 2.65–2.55 (m, 4 H), 1.65-1.56 (m, 4 H), 1.49-1.40 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 155.58, 145.15, 141.27, 130.19, 129.37, 128.79, 127.71, 126.71, 123.68, 63.38, 54.94, 25.95, 24.39, 15.55; HRMS (ESI) calcd for (M+H) $C_{16}H_{19}ClN_2$: 275.1310, found 275.1301.



4.5

***N*-((3-chloro-4-methylquinoline-2-yl)methyl)-*N*-ethylethanamine (4.5)** To a solution of acetonitrile (10 mL), **4.1** (1.245, 5.50 mmol), and potassium carbonate (0.761 g, 5.50 mmol) was added pyrrolidine (1.14 mL, 11.01 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **4.5** (1.168 g, 81%) as a white oil: R_f 0.23 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.09 (dd, 1 H, *J* = 1.0, 7.6 Hz), 7.96 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.67 (td, 1 H, *J* = 1.2, 7.2 Hz), 7.55 (td, 1 H, *J* = 1.2, 7.2 Hz), 4.01 (s, 2 H), 2.77 (s, 3 H), 2.74 (q, 4 H, *J* = 7.2 Hz), 1.09 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 156.4, 145.18, 141.30, 130.14, 129.22, 128.77, 127.74, 126.67, 123.69, 58.09, 47.10, 15.50, 11.40; HRMS (ESI) calcd for (M+H) $C_{15}H_{19}ClN_2$: 263.1310, found 263.1302.

4.4 References for Chapter 4

(1) Degtyarenko, A. S.; Tolmachev, A. A.; Volovenko, Y. M.; Tverdokhlebov, A. V. *Synthesis*. **2007,** *24*, 3891-3895.

(2) Friedlaender, P. *Eur. J. Inorg. Chem.* **1882,** *5*, 2572-2575.

(3) Ryabukhin, S. V.; Naumchik, V. S.; Plaskon, A. S.; Grygorenko, O. O.; Tolmachev, A. A. *J. Org. Chem*. **2011,** *76*, 5774-5781.

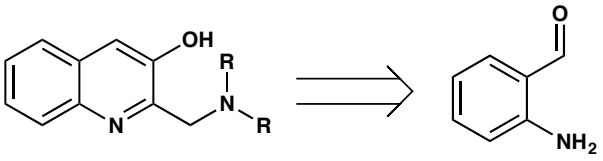
CHAPTER FIVE

SYNTHESIS OF 3-HYDROXY-2-(AMNIOMETHYL)QUINOLINE

5.1 Introduction

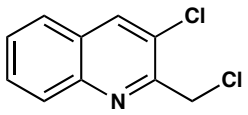
The current lead compound framework is based on 3-hydroxy-2-(aminomethyl)pyridine. Molecules in this library of QMPs have shown the most promise in their alkylating abilities by showing the highest percentage of conversion of aged AChE to its active form. Computationally, it has shown that adding another ring to the aromatic system and increasing its hydrophobicity makes it a stronger binder in the active site of AChE. This improved binding energy could be a significant indicator that with this favored set of interaction could lead to this compound being a good alkylator of aged AChE.

In the previous chapter, 2-aminoacetophenone was used to generate a quinoline framework similar to the one desired, except with a methyl group at the three position. It is our hope that by using 2-aminobenzaldhyde, the quinoline framework can be generated without the methyl group (Scheme 5.1). The reason for not using this compound previously is due to its expensive nature. However, it is necessary in order to compare to our lead compound. Our goal is to find a way to synthesize the di-halogenated compound. Once this compound is synthesized with two halogens, a S_N2 and NAS could be performed to make the desired QMP libraries, like in Scheme 2.2.



Scheme 5.1: Retrosynthesis of 2-hydroxy-3-(aminomethyl)quinoline from 2-aminobenzophenone

Having the ability to vary the leaving group by performing a S_N2 allows for a wide variety of nucleophiles to be placed onto the quinoline framework and respectively tested for their ability to resurrect the aged enzyme (Figure 5.1). This will allow us to screen a variety of compounds to accurately hone in one what makes a compound a significant realkylator of the enzyme.



5.1

Figure 5.1: Desired dihalogenated quinoline to generate a QMP library

This chapter will discuss the synthesis of a library of QMPs based on the general framework of 2-hydroxy-3-(aminomethyl)quinoline.

5.2 Results, Discussion, and Conclusions

As discussed in the previous chapter, 2-aminoacetophenone is a commonly used reagent in the literature for the generation of substituted quinoline frameworks. However, 2-aminobenzaldehyde is not as common reagent in these literature sources. For example, the same paper that cites the use of 2-aminoacetophenone and TMSCl to synthesize substituted quinolines has no mention of aldehyde in the paper, solely ketones[1]. The few literature sources found that use 2-aminobenzaldehyde employ a variety of expensive catalysts, like metals and ionic liquids, to form the quinoline. The

quinoline was attempted to be generated from the same conditions used to make 3-chloro-2-(chloromethyl)-4-methylquinoline (Figure 4.2).

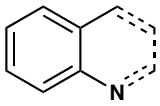
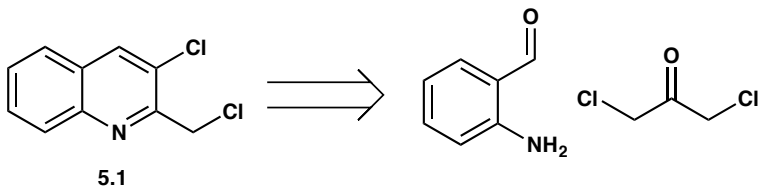


Figure 5.2: Use of 2-aminobenzaldehyde to synthesize quinoline framework

The synthesis followed originally used 2-aminoacetophenone and 1,3-dichloroacetone with TMSCl as a catalyst to generate the quinoline framework (Scheme 4.6). The same reaction scheme was used with all of the same starting materials, except replacing 2-aminoacetophenone with 2-aminobenzaldehyde. The methyl group on 3-chloro-2-(chloromethyl)-4-methylquinoline came from the ketone off of 2-aminoacetophenone, as discussed in the mechanism in Scheme 4.5. This would enable the same ketone, 1,3-dichloroacetone, to be the other starting material used in the reaction to generate the desired quinoline (Scheme 5.2).

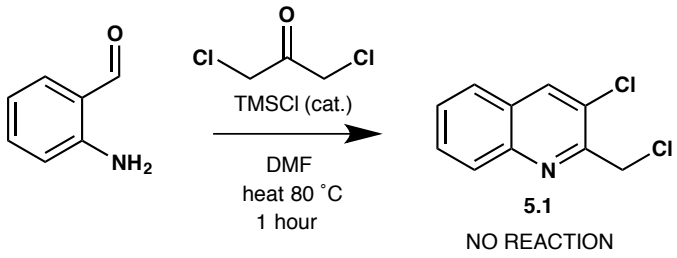


5.1

Scheme 5.2: Retrosynthetic analysis of the formation of **5.1** from 2-aminobenzaldehyde and 1,3-dichloroacetone

Unfortunately, with the use of 2-aminobenzaldehyde, no product formation was observed after heating for 1 hour, like there was in the previous procedure (Scheme

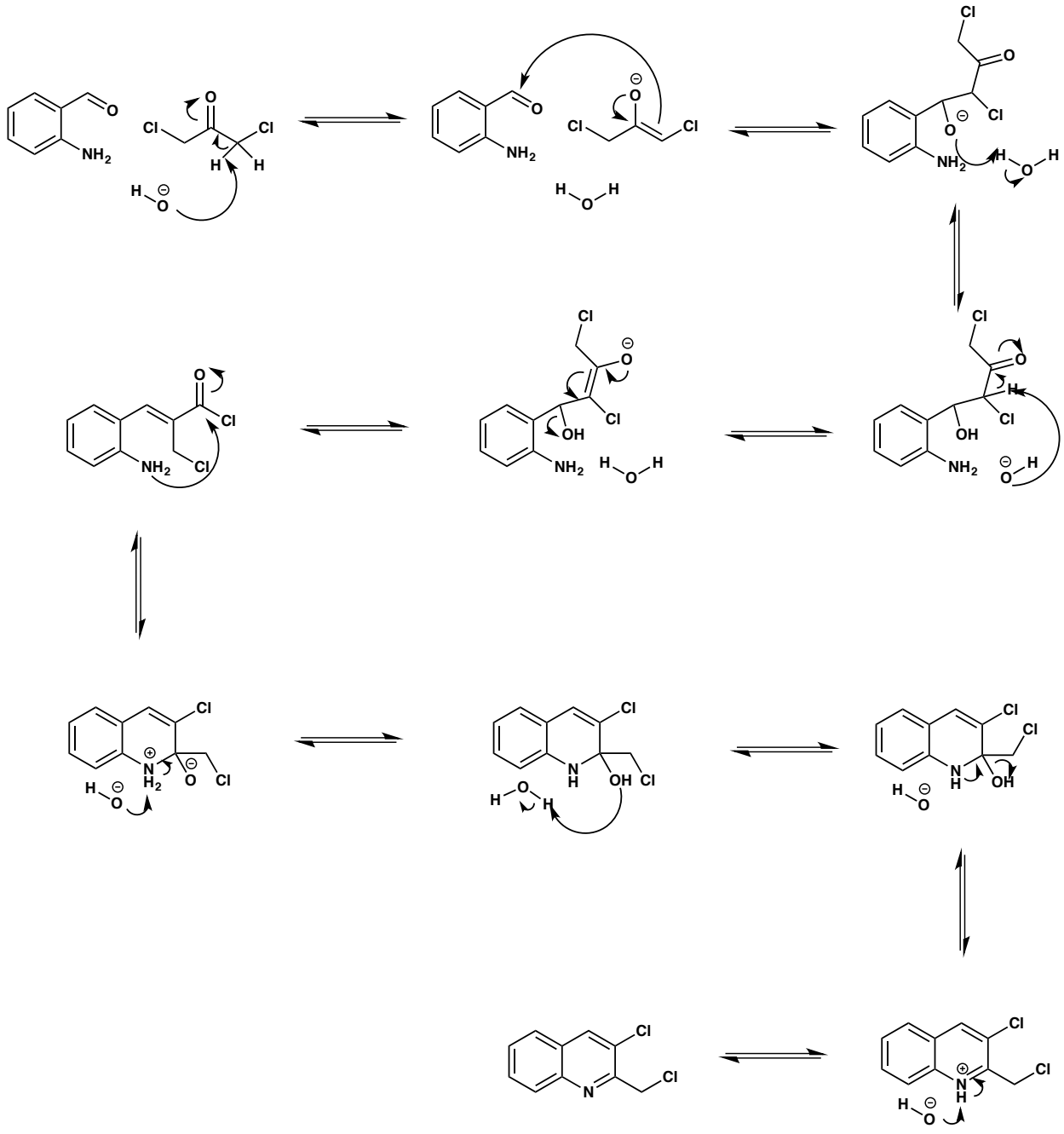
5.3). As noted, the paper cited no use of aldehydes in these conditions. The carbonyl carbon of the aldehyde is more electron deficient than the ketone. Under the procedure previously discussed, the aldehyde could be susceptible to attack from other nucleophiles in the system before the enamine addition. Regardless, there reaction conditions were not able to form the product, and other conditions were sought to be able to use the same starting materials to generate the final product.



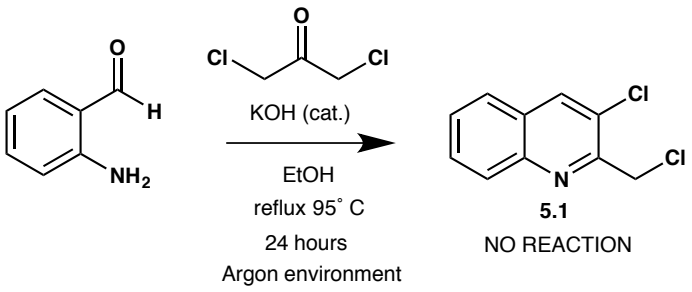
Scheme 5.3: Attempted synthesis of **5.1** from 2-aminobenzaldehyde with a TMSCl catalyst

Friedlaender Annulation syntheses are commonly performed in basic conditions like potassium hydroxide. The literature cited the use of potassium hydroxide worked well to generate quinolines from aldehyde derivatives[2]. By switching from acid-catalyzed to base-catalyzed, the hope is that the reaction is able to form the desired product and overcome the activation energy barrier to do so. The common mechanism for a base-catalyzed Friedlaender synthesis with an aldehyde is shown in Scheme 5.4. Since the aldehyde is more electrophilic, it should be more susceptible to nucleophilic attack by the enolate species. The reaction was first attempted in an Argon environment in ethanol under reflux for 24 hours (Scheme 5.5). The literature source for these

conditions did not contain the same substrates, but ones similar to it. After 24 hours, no product formation was observed.

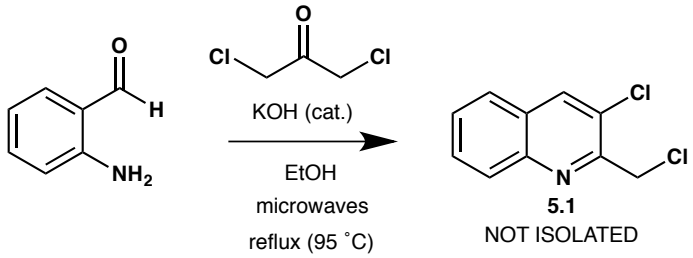


Scheme 5.4: General hydroxide-catalyzed mechanism for the cyclocondensation of 2-aminobenzophenone and 1,3-dichloroacetone to form quinoline



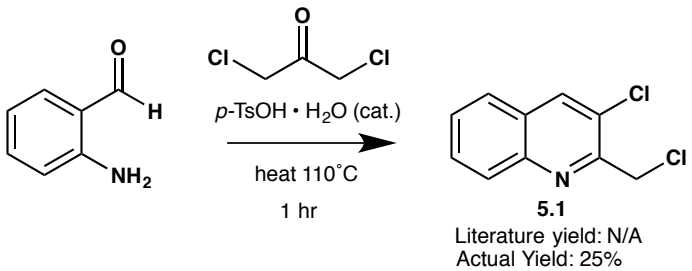
Scheme 5.5: Attempted synthesis of **5.1** from 2-aminobenzaldehyde with potassium hydroxide as the catalyst

The literature source cited that the same reaction could be conducted for shorter periods of time under microwave conditions[2]. The reaction was repeated in a microwave, instead of at reflux, for one hour to see if the microwave radiation could overcome the activation barrier (Scheme 5.6). The microwave conditions were set to 220 W at 95 ˚C for 1 hour. After 2 minutes in the microwave, desired product formation was visualized, but already contained side products. After the reaction time of 1 hour, multiple side products were observed. Mass spectrometric analyses were conducted and showed multiple products with one mass matched the expected mass for product **5.1**. The small amount of product formed was not able to be isolated under these conditions, and alternate conditions were again sought out to catalyze this conversion.



Scheme 5.6: Attempted synthesis of **5.1** from 2-aminobenzaldehyde using a microwave under base-catalyzed conditions

A paper reported the use of solvent-free conditions to catalyze the conversion of the aldehyde derivative into the quinoline framework[3]. Once again, the source did not contain the exact reagents or product, but used similar frameworks containing aldehydes. The paper cited a list of catalysts they attempted in achieving this conversion, and the one that showed potential was *para*-toluene sulfonic acid (*p*-TsOH). By using the monohydrate (*p*-TsOH•H_2O) the conversion can be accomplished without the need for solvent. The reagent is commercially available and inexpensive, and has often been used to catalyze the formation of a variety of heterocyclic compounds. The paper cited the use of either microwave or conventional heating to catalyze the conversion from various amino-benzaldehyde compounds. The reactions mix as they melt at these high temperatures and can effectively react. The microwave data showed faster reaction times, on the order of seconds rather than minutes, but the convenience of conventional heating won out and was attempted first to see if the conditions could work to form the desired product, **5.1** (Scheme 5.7). The mechanism is similar to that observed under base-catalyzed conditions. This mechanism is standard for Friedlaender Annulation reactions.



Scheme 5.7: Synthesis of **5.1** from 2-aminobenzaldehyde and 1,3-dichloroacetone

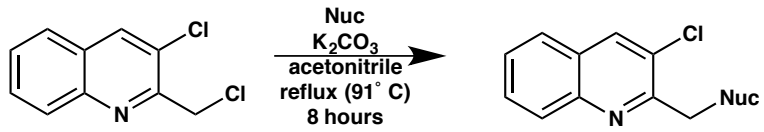
The paper cites yields of similar compounds in the order of 85-100% yield. However, the yield for this reaction with our reagents was only 25%. For true comparison, the paper did not use the same 1,3-dichloroacetone reagent or the same substrate. Their amino substrates were more electron deficient, and their ketone compound was more electron rich than the ones used in Scheme 5.7. The solid product proved impossible to separate from the multitude insoluble salts, so column chromatography is required to just be able to observe the product.

The microwave conditions used in the paper were attempted for 1 minute at 100 ˚C at 300 W. The reaction yielded many products, so the conventional heating conditions were deemed better to form **5.1**. The yield was sufficient enough to generate a small amount of starting material to avoid buying lots of material. Other reaction conditions can be sought to make **5.1** in a higher yield and a lower cost if they prove to have strong ability to realkylate aged AChE.

The reaction to generate **5.1** seems highly dependent upon the reaction conditions. The catalyst plays a large part in the formation of this product. In the future we can screen a variety of conditions to optimize this reaction.

The general protocol of an S_N2 was performed on **5.1** to begin the synthesis of the library for this framework. The amines utilized on the framework were pyrrolidine, 2-methylpyrrolidine, piperidine, and diethylamine. These amines were shown to have the most promise on our current lead compounds, as the percent resurrection for these compounds on the pyridine framework were the highest seen for any of the compounds. By maintaining the same amine, one could analyze the sole effect of the addition of the extra ring system off the pyridine ring in the form of quinoline and its effects on its ability

to resurrect aged AChE. Since these four amines were the ones that showed activity, only these four were placed on the ring to see if the generated framework had any activity (Scheme 5.8).



5.1

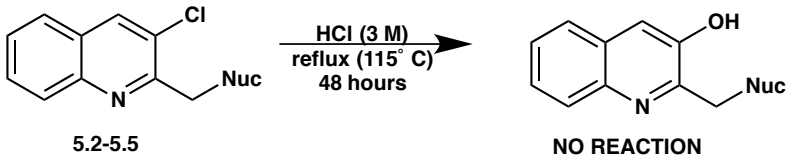
5.2, Nu = pyrrolidine, 100%
5.3, Nu = 2-methylpyrrolidine, 89%
5.4, Nu = piperidine, 88%
5.5, Nu = diethylamine, 45%

Scheme 5.8: S_N2 reaction of **5.1** and various amine nucleophiles

This framework is very adaptable due to having the chlorine on the primary position, which is prime placement for nucleophilic attack. If this framework does go on to show realkylating abilities, it is very easy to change the leaving group to a wide variety of different amines and other varied nucleophiles. This could further expand the library of QMPs and hone in what makes the framework an acceptable alkylator. The yields of the S_N2 reactions were quite varied, with a range of around 40-100%. It can be assumed the yield is normally 90-100% as seen for three of the frameworks, and the low yield for **5.5** can be attributed to human error. This was still high enough yield to synthesize the QMP.

The NAS reaction on the 3-chloro frameworks was discussed earlier in this thesis. The 3-chloro frameworks do not proceed easily under the same conditions that are successful for the 2-chloro frameworks. The NAS reaction was still attempted, but

no reaction occurred (Scheme 5.9). A hydroxyl group is needed to generate a library of QMPs, since the QM cannot form without the hydroxyl group.



5.2-5.5

NO REACTION

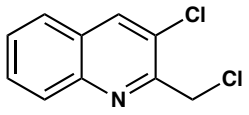
Scheme 5.9: Attempted NAS reaction of **5.2-5.5** with 3 M hydrochloric acid

Currently, there are no reactions that have been successful in converting **5.2-5.5** into their hydroxyl derivatives for a library of QMPs. However, these four compounds will be tested to see their ability to realkylate aged AChE to see how, even without the hydroxyl group, they compare to the lead compounds due to their similarly placed substituents. Perhaps, even though they cannot form a QM in the body, they can realkylate aged AChE through an alternate mechanism. Alternate reaction conditions to generate the desired product, 3-hydroxy-2-(methylamino)quinoline, from a different starting material will be discussed in the following chapter.

The Friedlaender Annulation reaction proves synthetically useful to generate substituted quinoline frameworks in high yields. The reaction can be useful in generating other QMP frameworks. The reaction is highly dependent upon the substrate(s) and the catalyst used. This method provides a semi-efficient, though semi-costly, way to synthesize the 3-chloro-2-(aminomethyl)quinoline compounds, though not yet the desired QMP compounds until conditions to convert the chlorine to a hydroxyl group are discovered.

5.3 Experimental Data

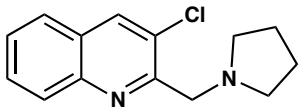
General Methods The solvents used in these experiments were not purified any further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



5.1

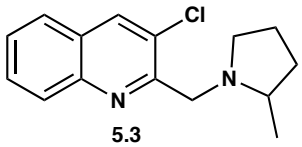
3-chloro-2-(chloromethyl)-quinoline (5.1) To a solution of 2-aminoacetophenone (0.250 g, 2.06 mmol), and 1,3-dichloroacetone (0.262 g, 2.06 mmol) was added *p*-TsOH•H_2O (0.393, 2.06 mmol). The reaction mixture was heated to 110 °C for 1 h. The reaction was cooled to rt and then diluted with water and neutralized with sodium hydroxide (10%). The reaction was extracted with dichloromethane, and the organic

layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (10:1 hexanes:EtOAc) to yield **5.1** (0.110 g, 25%) as a white powdery solid: R_f 0.50 (10:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.21 (d, 1 H, J = 1.2 Hz), 8.11 (dd, 1 H, J = 1.2, 8.0 Hz), 7.80–7.71 (m, 2 H), 7.60 (td, 1 H, J = 1.2, 8.0 Hz), 4.97 (s, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 153.52, 146.05, 135.18, 130.10, 129.44, 128.58, 128.13, 127.89, 126.62, 45.01; HRMS (ESI) calcd for (M+Na) $C_{10}H_7Cl_2N$: 233.9848, found 233.9837.



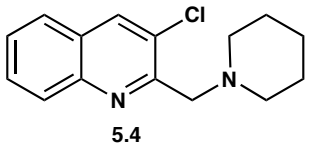
5.2

3-chloro-2-(pyrrolidin-1-ylmethyl)quinoline (5.2) To a solution of acetonitrile (10 mL), **5.1** (0.096 g, 0.452 mmol), and potassium carbonate (0.063 g, 0.452 mmol) was added pyrrolidine (0.08 mL, 0.905 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **5.2** (0.115 g, 100%) as a yellow oil: R_f 0.17 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.15–8.10 (m, 2 H), 7.77–7.65 (m, 2 H), 7.52 (td, 1 H, J = 1.2, 8.4 Hz), 4.10 (s, 2 H), 2.80–2.72 (m, 4 H), 1.98–1.89 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 156.10, 146.07, 135.10, 129.55, 129.34, 128.45, 127.83, 127.02, 126.47, 59.50, 54.49, 23.60; HRMS (ESI) calcd for (M+H) $C_{14}H_{15}ClN_2$: 247.7455, found 247.0984.



5.3

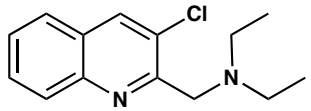
3-chloro-2-((2-methylpyrrolidin-1-yl)methyl)quinoline (5.3) To a solution of acetonitrile (10 mL), **5.1** (0.079 g, 0.373 mmol), and potassium carbonate (0.052 g, 0.373 mmol) was added pyrrolidine (0.08 mL, 0.745 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **5.3** (0.086 g, 89%) as a light orange powdery solid: R_f 0.38 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.15–8.10 (m, 2 H), 7.78–7.65 (m, 2 H), 7.53 (td, 1 H, *J* = 1.2, 8.4 Hz), 4.48 (d, 1 H, *J* = 13.2) 3.62 (d, 1 H, *J* = 13.2), 3.21–3.12 (m, 1 H), 2.67–2.56 (m, 1 H), 2.39 (q, 1 H, *J* = 9.2), 2.01–1.91 (m, 1 H), 1.82–1.60 (m, 2 H), 1.58–1.45 (m, 1 H), 1.24 (d, 1 H, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 156.57, 145.95, 135.29, 129.45, 129.32, 128.85, 127.94, 127.02, 126.49, 60.45, 57.84, 54.43, 32.62, 21.82, 19.14; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1080, found 261.1142.



5.4

3-chloro-2-(piperidine1-ylmethyl)quinoline (5.4) To a solution of acetonitrile (10 mL), **5.1** (0.076 g, 0.358 mmol), and potassium carbonate (0.050 g, 0.358 mmol) was added

pyrrolidine (0.07 mL, 0.717 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **5.4** (0.082 g, 88%) as a light yellow powdery solid: R_f 0.35 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.15–8.09 (m, 2 H), 7.77–7.65 (m, 2 H), 7.52 (td, 1 H, *J* = 1.2, 8.4 Hz), 3.89 (s, 2 H), 2.68–2.54 (m, 4 H), 1.68–1.55 (m, 4 H), 1.48–1.40 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 156.88, 145.82, 135.29, 129.48, 129.35, 129.28, 127.88, 127.07, 126.48, 62.48, 54.85, 25.95, 24.36; HRMS (ESI) calcd for (M+H) $C_{15}H_{17}ClN_2$: 261.1153, found 261.1144.



5.5

***N*-((3-chloro-quinoline-2-yl)methyl)-*N*-ethylethanamine (5.5)** To a solution of acetonitrile (10 mL), **5.1** (0.092, 0.443 mmol), and potassium carbonate (0.060 g, 0.443 mmol) was added pyrrolidine (0.08 mL, 0.868 mmol). The reaction mixture was refluxed for 8 h. The reaction was diluted with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (2:1 hexanes:EtOAc) to yield **5.5** (0.048 g, 45%) as a brown oil: R_f 0.39 (9:1, CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.15–8.09 (m, 2 H), 7.77–7.65 (m, 2 H), 7.52 (td, 1 H, *J* = 1.2, 8.4 Hz), 4.01 (s, 2 H), 2.73 (q, 4 H, *J* = 7.2 Hz), 1.09 (t, 6 H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C)

156.88, 145.82, 135.29, 129.48, 129.35, 129.28, 127.88, 127.07, 126.48, 62.48, 54.85, 25.95, 24.36; HRMS (ESI) calcd for (M+H) $C_{14}H_{17}ClN_2$: 249.1080, found 249.1146.

5.4 References for Chapter 5

(1) Degtyarenko, A. S.; Tolmachev, A. A.; Volovenko, Y. M.; Tverdokhlebov, A. V. *Synthesis* **2007,** *24*, 3891-3895.

(2) Li, A. D.; Beard, I.; Coate, H.; Hona, A.; Kadalbajoo, M.; Kleinberg, A.; Laufer, R.; Mulvihill, K. M.; Nigro, A.; Rastogi, P.; Sherman, D.; Siu, K. W.; Steinig, A. G.; Wang, T.; Werner, D.; Crew, A. P.; Mulvihill, M. T. *Synthesis* **2010,** *10*, 1678-1686.

(3) Jia, C.; Zhang, Z.; Tu, S.; Wang, G. *Org. and Biomol. Chem*. **2006,** *4*, 104-110.

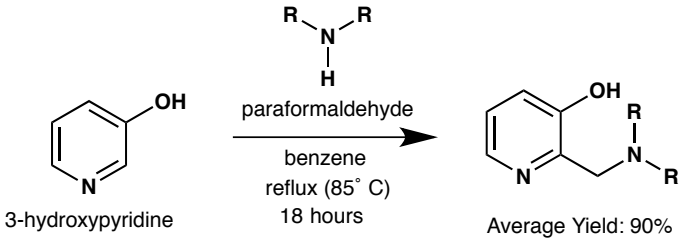
CHAPTER SIX

MANNICH REACTIONS FOR THE SYNTHESIS OF (AMINOMETHYL)QUINOLINOLS

6.1 Introduction

As mentioned previously, the current lead compound is based on 3-hydroxy-2-(aminomethyl)pyridine, as molecules with this general framework have had the highest percentages of resurrection of aged acetylcholinesterase. The quinoline version of this framework, 3-hydroxy-2-(aminomethyl)quinoline, is consequently highly desired for screening. Computationally, the extra ring has shown to have favorable pi electron interactions in the active site and binds more favorably compared to its pyridine counterpart.

Other attempts at this framework were discussed in chapter five of this thesis, but none proved promising to synthesize the desired quinoline. The pyridine framework itself was synthesized using a Mannich reaction (Scheme 6.1). The reaction started with 3-hydroxypyridine and utilized paraformaldehyde and a secondary amine to add the aminomethyl group to the two position of the pyridine ring.



Scheme 6.1: Synthesis of 3-hydroxy-2-(aminomethyl)pyridine via a Mannich reaction with 3-hydroxyquinoline

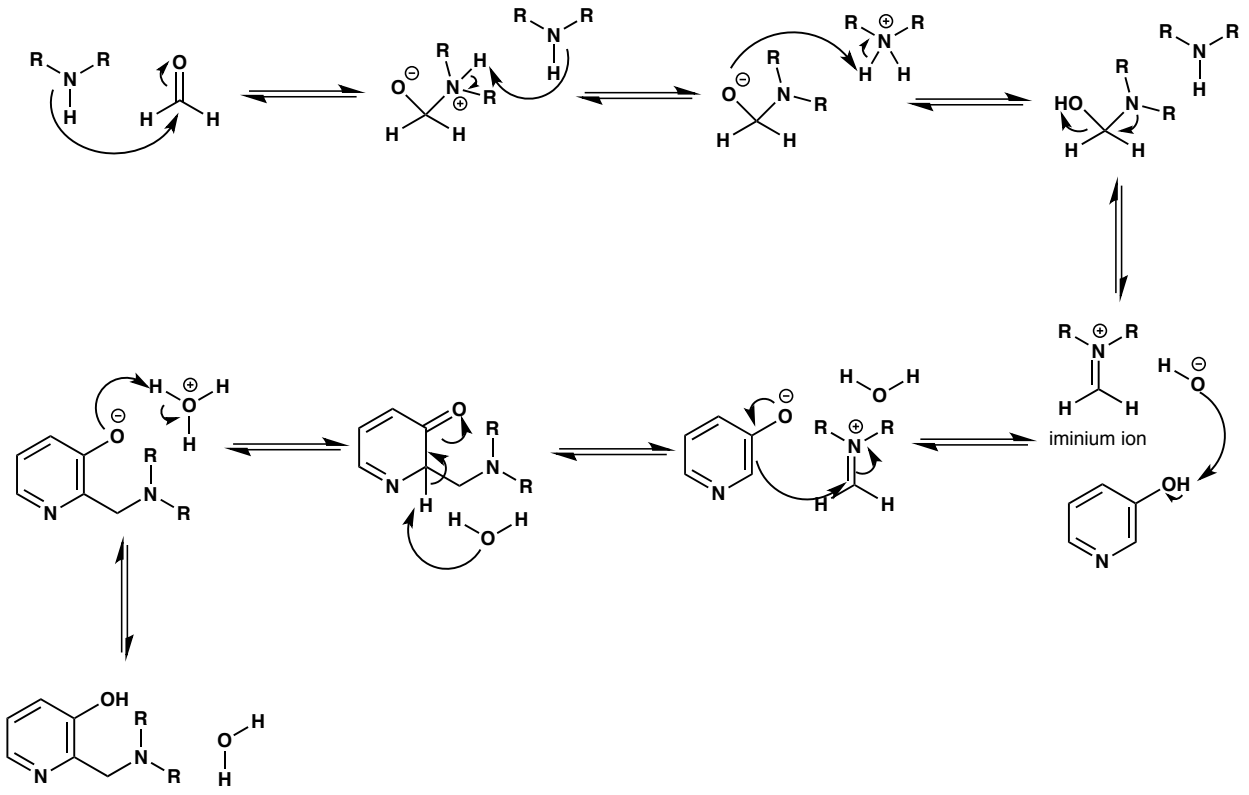
The next attempt at the synthesis of the quinoline framework therefore was to use these Mannich reactions to add the desired amine groups to the framework of

various quinolinols, specifically 3-hydroxyquinoline. Though this framework does not have the halogen substituents seen on the previous frameworks in this thesis, the amine group can still be varied to allow a variety of amines to be placed onto the ring in order to see how each affects the compound's ability to realkylate aged acetylcholinesterase. Mannich conditions are also inexpensive and readily available, making them desirable conditions to be able to synthesize a large library with.

This chapter will discuss the synthesis of a library of QMPs based on frameworks synthesized through Mannich reactions.

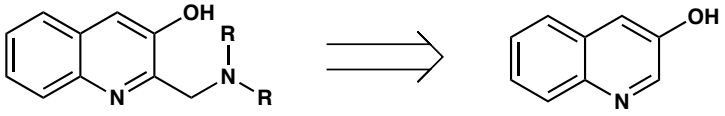
6.2 Results, Discussion, and Conclusions

The Mannich reaction is typically used in the literature to convert a primary or secondary amine, a non-enolizable carbonyl, and an enolizable carbonyl to a β-carbonyl compound. The final product is commonly referred to as the Mannich base[1]. In the mechanism for the Mannich reaction with 3-pyridinol, the pyridinol serves as the enol without the need to tautomerize, and the product reforms this enol species in order to retain aromaticity in the product. Neither acid nor base was used to catalyze the reaction (Scheme 6.2). The secondary imine and paraformaldehyde react first to form an iminium ion through a tetrahedral intermediate, followed by the elimination of water. The iminium ion goes on to react with the pyridine compound, getting attacked by the enol pi electrons to form a carbonyl intermediate. The compound then reforms aromaticity to form the desired 3-hydroxy-2-(aminomethyl)pyridine. This mechanism was assumed to be the consistent for quinolinol compounds as well with the use of secondary amines and paraformaldehyde.



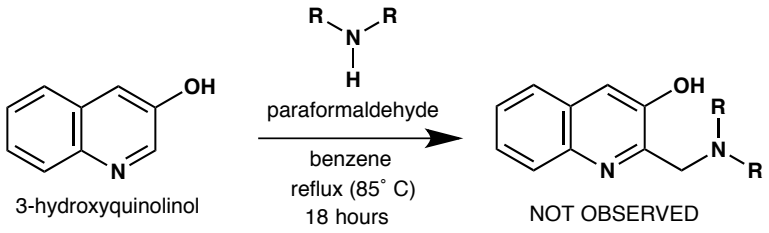
Scheme 6.2: General Mannich reaction mechanism with a secondary amine, paraformaldehyde, and 3-pyridinol to form 3-hydroxy-2-(aminomethyl)pyridine

The quinolinol that was used with these same Mannich conditions was the 3-hydroxy-2-(aminomethyl)quinoline compound, in order to make the desired quinoline framework to directly compare it to the lead pyridine compound. The reagent that was purchased to perform a Mannich reaction on was 3-hydroxyquinoline, since the similar pyridine framework was made from 3-hydroxypyridine (Scheme 6.3).



Scheme 6.3: Retrosynthetic analysis of the formation of 3-hydroxy-2-(aminomethyl)quinoline from 3-hydroxyquinoline

The literature source that has the pyridine Mannich reactions also describes the use of various quinolines with the same reaction conditions. The paper used a one-pot method with an aprotic solvent in order to form the aminomethyl on the quinoline compounds[2]. However, the literature source did not use 3-quinolinol in its substrate scope, only 4-quinolinol and 8-quinolinol. Both of those substrates were reported with high yields, so the Mannich reaction on 3-quinolinol was attempted. As for 3-pyridinol, the aminomethyl group was expected to add into the two position of the quinoline ring, *ortho* to both the quinoline nitrogen and the hydroxyl group. This expected product, however, was not observed (Scheme 6.4). The ^{1}HNMR still showed the quinoline peaks and the expected methylene peak, but the aromatic region did not match the expected compound, but instead looked to be an isomer of it.



Scheme 6.4: Attempted synthesis of 3-hydroxy-2-(aminomethyl)quinoline from 3-hydroxyquinoline

A source found in the literature analyzed the most reactive positions of pyridines and quinolines for Mannich reactions[3]. The source stated that 3-pyridinol does indeed direct to the two position of the framework for aminomethylation. However, 3-quinolinol, like 3-naphthalenol, was found to direct to the four position of the ring, not the two position. The literature source cited evidence solely based on the shift of the methylene

peak, so we conducted further analysis on this framework to see if this statement was correct.

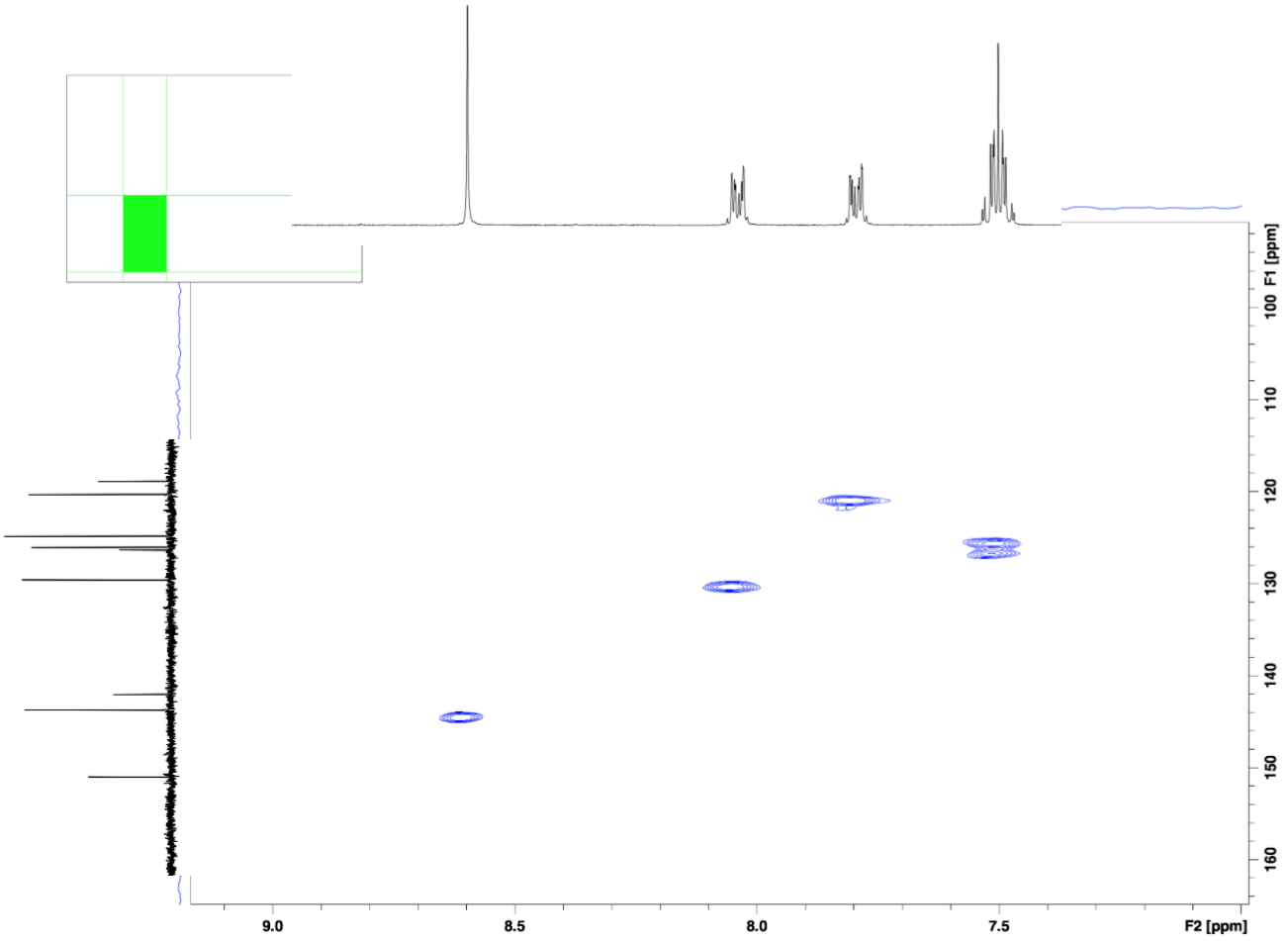


Figure 6.1: HMQC data for the product from Scheme 6.4, with ^{1}HNMR and ^{13}CNMR data

An analysis was performed with 2D ^{1}HNMR, specifically HMQC (Figure 6.1). The most deshielded proton was found to couple to the most deshielded carbon that was bonded to a proton. If the compound was matched what was expected, the highest shifted proton would be expected to couple to a carbon with a lower shift value. The

highest proton for quinoline would be the one *ortho* to the oxygen group. If the aminomethyl were on the 2-position, the only proton *ortho* would be on the 4-position. The carbon on position four is found to be one of the most upfield shifted carbon atoms. However, in Figure 6.1, it is seen that the highest proton is coupled to one of the highest carbons. The carbon it is coupling to is the carbon that is *ortho* to both the hydroxyl group and the nitrogen in the ring. Therefore, there is a proton at the 2-position of this quinoline framework. By analyzing the HMQC, it can be confidently stated that the aminomethyl group is indeed on the four position of the quinoline ring.

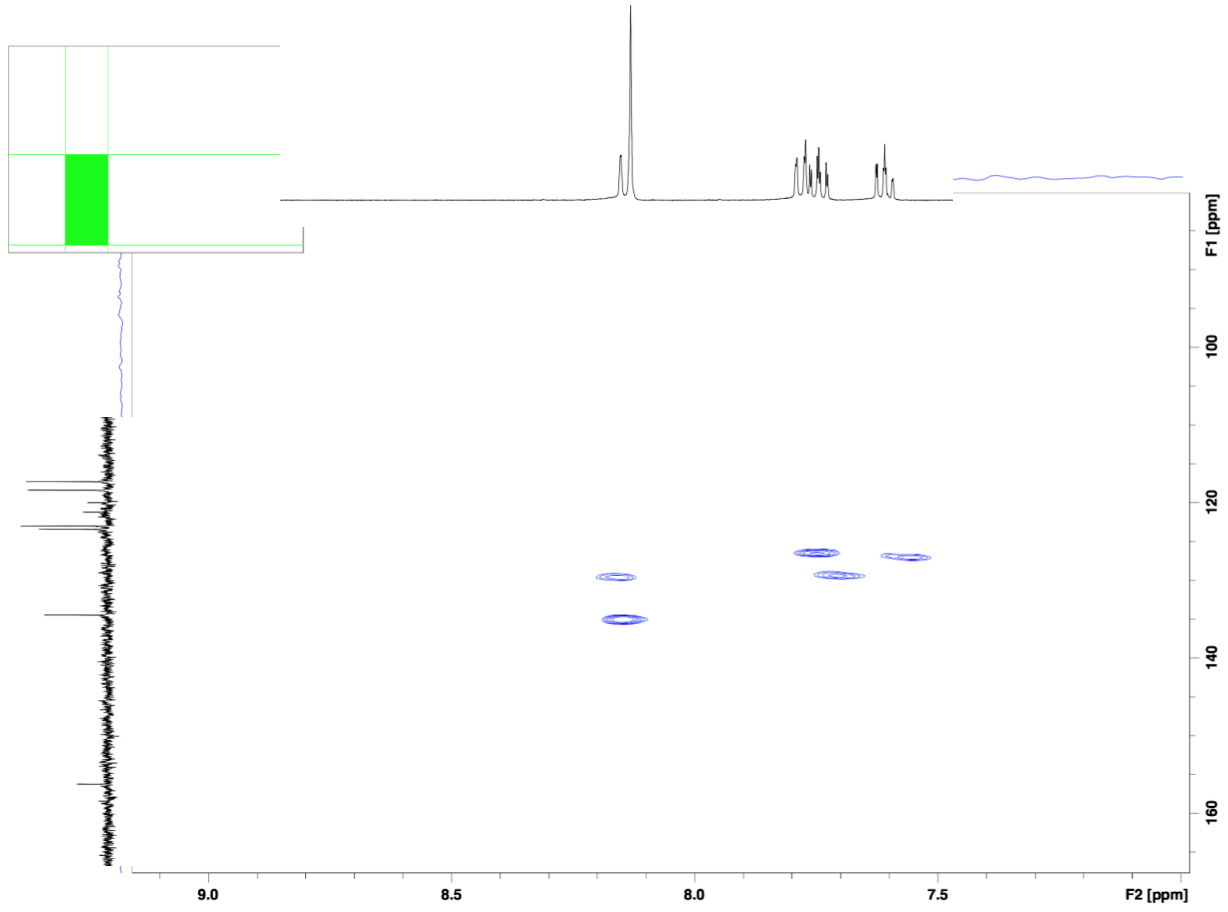
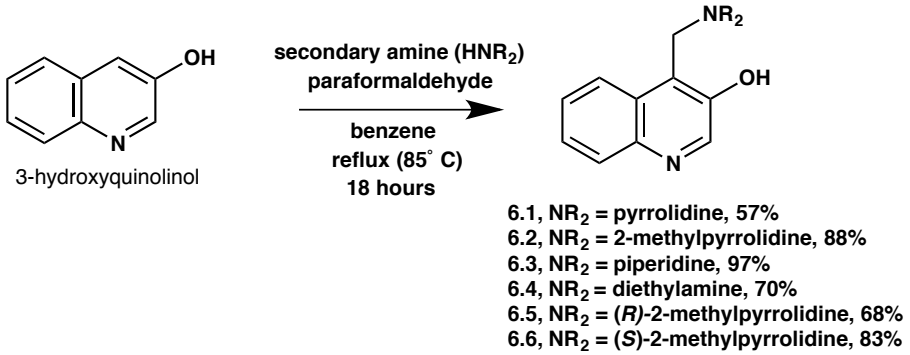


Figure 6.2: HMQC data for **5.2**, with ^{1}HNMR and ^{13}CNMR data

A similar HMQC analysis was performed on compound **5.2**, 3-chloro-2-(pyrrolidin-1-ylmethyl)quinoline, which showed that the proton associated with the 4-position of the ring couples to a much lower carbon around 129 ppm, than the one in the 2-position, which couples to a carbon around 142 ppm (Figure 6.2). It can be determined that the framework generated is in fact 3-hydroxy-4-(aminomethyl)quinoline, rather than 3-hydroxy-2-(aminomethyl)quinoline.

The protocol in Scheme 6.4 was followed with four amines, pyrrolidine, 2-methylpyrrolidine, piperidine, and diethylamine (Scheme 6.5). These amines were shown to have the highest percent resurrection of aged acetylcholinesterase on the pyridine framework. The (*R*) and (*S*) enantiomers of 2-methylpyrrolidine were also synthesized for testing. By analyzing the same set of leaving groups, it will allow us to provide a comparison between the two frameworks. By maintaining the same amine, one can analyze the sole affect of the addition of extra ring in quinoline system compared to pyridine.

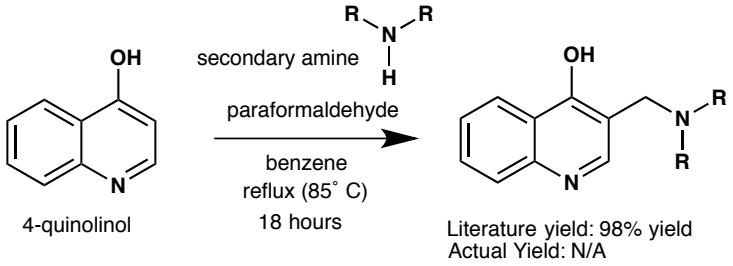


Scheme 6.5: Mannich reaction of 3-hydroxyquinolinol and various secondary amines

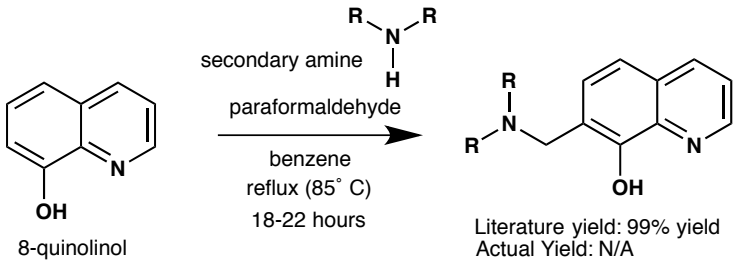
This is the first quinoline compound synthesized with the hydroxyl group in the

3-position of the ring. Though the leaving group is not in the same position as the lead compound, computationally the compound was suggested to work at high percentages as well. The Mannich reaction afforded yields between 57% and 97%. This wide range can be potentially attributed to the varied reactivity of the various iminium ions and the varied rates the amines in forming the iminium ion. The yields were suitable in producing enough to use to perform kinetic tests on, especially since the reagents were available inexpensively and could be run on larger scales. The screening results of compounds **6.1-6.6** will be discussed later on in this thesis.

The paper reported the use of 4-quinolinol and 8-quinolinol under the same Mannich reaction conditions in extremely high yields. Following the same procedure as for compounds **6.1-6.6**, no reaction occurred with either substrate (Schemes 6.6 and 6.7).



Scheme 6.6: Attempted Mannich reaction with 4-quinolinol



Scheme 6.7: Attempted Mannich reaction with 8-quinolinol

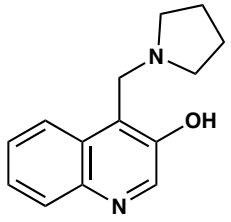
The reaction is the same as the 3-quinolinol compound, so it was unexpected that it would not work. The paper reported that the reactions are highly sensitive to solvent. A protic solvent instead should be attempted next to see if that could help the reaction proceed to be able to make other useful quinolinol frameworks with these Mannich conditions.

The Mannich reaction proves synthetically usefully in generating substituted quinolinol frameworks in high yields. Though only one framework was generated, the reaction can hopeful be used in the future to synthesize other QMP frameworks as well. The reaction is cost efficient and provides high yields and is an affective way to place various leaving groups onto the 3-quinolinol framework in the 4-position. In order to obtain the desired 3-hydroxy-2-(aminomethyl)quinoline framework, alternate conditions aside from the Mannich ones would need to be used. This method provides an efficient way to synthesize a 3-hydroxy-4-(aminomethyl)quinoline library.

6.3 Experimental Data

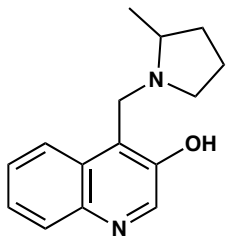
General Methods The solvents used in these experiments were not purified any further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using

Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



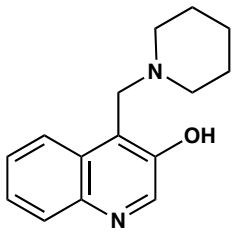
6.1

2-(pyrrolidin-1-ylmethyl)quinolin-3-ol (6.1) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.35 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.1** (0.448 g, 57%) as a brown oil: R_f 0.11 (1:2, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.58 (s, 1 H), 8.03 (dd, 1 H, J = 2.0, 8.4 Hz), 7.79 (dd, 1 H, J = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.30 (s, 2 H), 2.85–2.71 (m, 4 H), 1.98–1.89 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.84, 144.51, 142.83, 130.30, 127.02, 126.74, 125.51, 120.93, 119.52, 54.20, 53.76, 23.74; HRMS (ESI) calcd for (M+H) $C_{14}H_{16}N_2O$: 229.1335, found 229.1352.



6.2

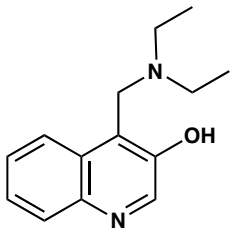
2-((2-methylpyrrolidin-1-yl)methyl)quinolin-3-ol (6.2) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.35 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.2** (0.734 g, 88%) as an orange oil: R_f 0.23 (1:2, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.56 (s, 1 H), 8.03 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.78 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.42 (d, 1 H, *J* = 15.6) 4.12 (d, 1 H, *J* = 15.6), 3.22–3.14 (m, 1 H), 2.78–2.68 (m, 1 H), 2.35 (q, 1 H, *J* = 8.4), 2.18–2.09 (m, 1 H), 1.95–1.88 (m, 2 H), 1.66–1.54 (m, 1 H), 1.29 (d, 1 H, *J* = 15.7); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.77, 144.53, 142.81, 130.32, 127.06, 126.73, 125.49, 120.91, 119.77, 60.78, 54.85, 52.00, 32.65, 21.87, 18.76; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1482.



6.3

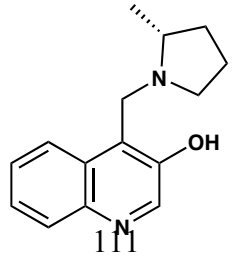
2-(piperidin-1-ylmethyl)quinolin-3-ol (6.3) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.34 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.3** (0.810 g, 97%) as a yellow oil: R_f

0.34 (1:2, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.57 (s, 1 H), 8.03 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.76 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.12 (s, 2 H) 3.08–2.50 (m, 4 H), 1.78–1.67 (m, 4 H), 1.70–1.39 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.87, 144.52, 142.87, 130.33, 127.40, 126.74, 125.51, 120.90, 118.75; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1808.



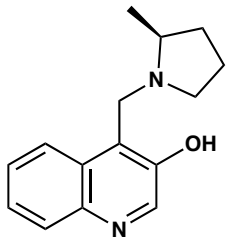
6.4

2-((diethylamino)-methyl)quinolin-3-ol (6.4) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.42 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.4** (0.556 g, 70%) as a red oil: R_f 0.30 (1:2, hexanes:EtOAc); ^{1}H NMR 400 MHz, $CDCl_3$, δ_H) 8.56 (s, 1 H), 8.02 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.76 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.22 (s, 2 H) 2.72 (q, 4 H, *J* = 14.4 Hz), 1.18 (t, 6 H, *J* = 8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 155.22, 144.67, 130.36, 127.32, 126.76, 125.45, 120.75, 119.24, 52.15, 47.55, 11.37; HRMS (ESI) calcd for (M+H) $C_{14}H_{18}N_2O$: 231.1492, found 231.1482.



6.5

(*R*)-2-((2-methylpyrrolidin-1-yl)methyl)quinolin-3-ol (6.5) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.35 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.5** (0.568 g, 68%) as an red oil: R_f 0.23 (1:2, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.56 (s, 1 H), 8.03 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.78 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.42 (d, 1 H, *J* = 15.6) 4.12 (d, 1 H, *J* = 15.6), 3.22–3.14 (m, 1 H), 2.78–2.68 (m, 1 H), 2.35 (q, 1 H, *J* = 8.4), 2.18–2.09 (m, 1 H), 1.95–1.88 (m, 2 H), 1.66–1.54 (m, 1 H), 1.29 (d, 1 H, *J* = 15.7); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.77, 144.53, 142.82, 130.32, 127.06, 126.72, 125.48, 120.90, 119.76, 60.78, 54.84, 52.00, 32.65, 21.87, 18.75; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1486.



6.6

(*S*)-2-((2-methylpyrrolidin-1-yl)methyl)quinolin-3-ol (6.6) To a solution of benzene (5 mL), 3-hydroxyquinoline (0.500 g, 3.44 mmol), and paraformaldehyde (0.114 g, 3.79 mmol) was added pyrrolidine (0.35 mL, 4.13 mmol). The reaction mixture was refluxed for 18 h. The reaction was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes:EtOAc) to yield **6.6** (0.695 g, 83%) as an red oil: R_f 0.23 (1:2, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.56 (s, 1 H), 8.03 (dd, 1 H, *J* =

2.0, 8.4 Hz), 7.78 (dd, 1 H, *J* = 2.0, 8.4 Hz), 7.52–7.44 (m, 2 H), 4.42 (d, 1 H, *J* = 15.6) 4.12 (d, 1 H, *J* = 15.6), 3.22–3.14 (m, 1 H), 2.78–2.68 (m, 1 H), 2.35 (q, 1 H, *J* = 8.4), 2.18–2.09 (m, 1 H), 1.95–1.88 (m, 2 H), 1.66–1.54 (m, 1 H), 1.29 (d, 1 H, *J* = 15.7); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.77, 144.53, 142.81, 130.32, 127.06, 126.73, 125.49, 120.91, 119.77, 60.78, 54.85, 52.00, 32.65, 21.88, 18.76; HRMS (ESI) calcd for (M+H) $C_{15}H_{18}N_2O$: 243.1492, found 243.1479.

6.4 References for Chapter 6

(1) Mannich, C.; Krösche, W. *Arch. Pharm. Med. Chem.* **1912**, *250*, 647–667.

(2) Chi, K.; Ahn, Y. S.; Shim, K. T.; Park, T. H.; Ahn, J. S. *Bull Kor Chem Soc*. **1999**, *20*, 973-976.

(3) Smirnov, L.D.; Andronova, N. A.; Lezina, V. P.; Dyumaev, K. M *Russ Chem Bull*. **1970**, *19*, 2241-2243.

CHAPTER SEVEN

SYNTHESIS OF (AMINOMETHYL)ISOQUINOLINOLS: CURRENT AND FUTURE WORK

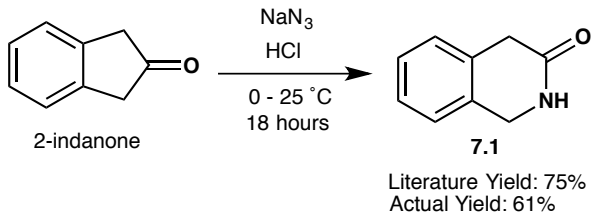
7.1 Introduction

Computationally, derivatives of isoquinolinol have shown promise in their ability to realkylate aged AChE. It has been shown to have closer proximity to the aged AChE in the active site due to the increased hydrophobicity and favorable interactions with nearby side chains. As previously discussed in this thesis, 3-hydroxy-2-(aminomethyl)pyridine has been the framework with the highest percent resurrection of AChE. Isoquinoline frameworks were sought out that could have similar substituents as this pyridine library.

Isoquinoline materials are not available commercially, and the very few that can be found are very expensive and not reasonable to use to generate a library of compounds. Reaction conditions were sought that could make substituted quinolines that could have an aminomethyl group and a hydroxyl group on the ring system. This chapter discusses the progress of these syntheses.

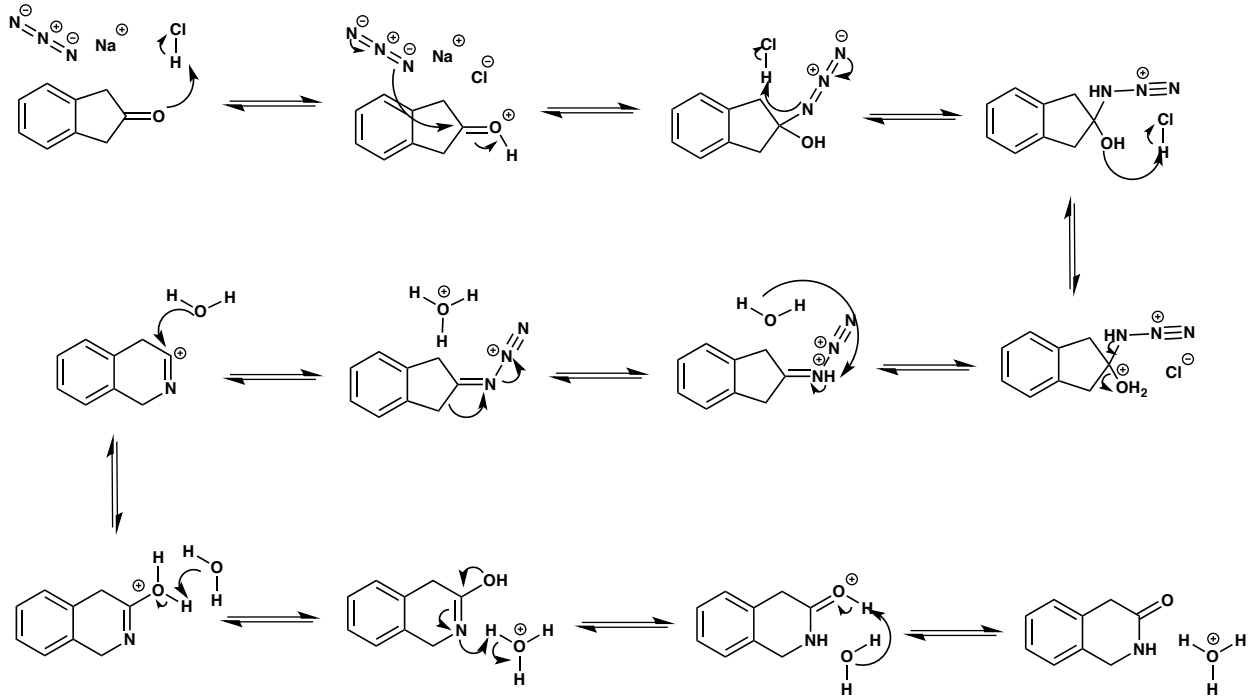
7.2 Results, Discussion, and Conclusion

A synthesis was found in the literature that used 2-indanone to generate an intermediate that could be used to form an isoquinoline framework that would be useful in generating a library of QMPs[1]. The first part of the reaction involves a ring rearrangement involving 2-indanone to form **7.1** (Scheme 7.1). The mechanism for the reaction is cited as the Schmidt rearrangement. The azide ion adds into the carbonyl via



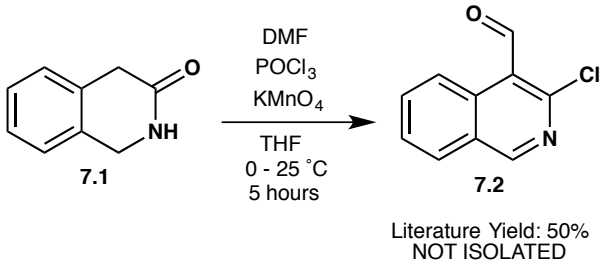
Scheme 7.1: Synthesis of **7.1** from 2-indanone and NaN_3

a tetrahedral intermediate and forms an imine like compound, which is extremely reactive. With the release of N_2, the ring rearranges, leaving a carbocation that is attacked by water and subsequently tautomerizes to form a carboxylic amide (Scheme 7.2).



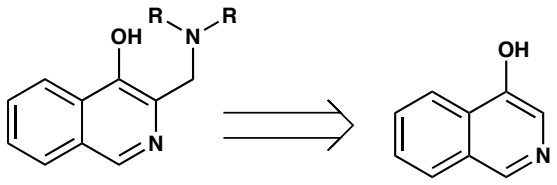
Scheme 7.2: Proposed mechanism for the formation of **7.2** from 2-inandone under acid-catalyzed conditions

The second part of the reaction involves a Vilsmeier-Haack reaction to form the substituted isoquinoline compound.[2] The Vilsmeier-Haack reaction was previously discussed in chapter three of this thesis, and is often used cited to add aldehydes to aromatic ring systems. The reaction uses the Vilsmeier reagent, made from DMF and $POCl_3$, along with $KMnO_4$ to produce the desired isoquinoline, **7.2**, from **7.1**. The aldehyde in the 4-position would allow for the placement of a variety of amine leaving groups through a reductive amination reaction. The framework has chlorine in the 3-position, which could be converted to the isoquinolinol compound through an NAS reaction. The literature reported a suitable yield of 50%. Unfortunately, the product was not able to be isolated (Scheme 7.3). The reaction produced many side products, and was difficult to purify, and after column chromatography only a few milligrams were able to be isolated. Alternate conditions were researched to have a more efficient synthesis for a library of QMPs. The reaction can be revisited in the future for optimization attempts.



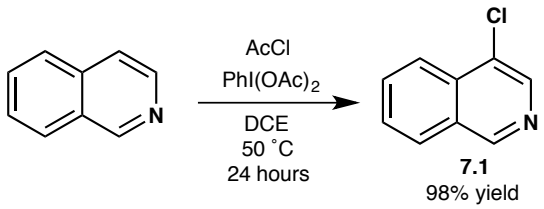
Scheme 7.3: Attempted synthesis of **7.2** from **7.1** though a Vilsmeier-Haack reaction along with $KMnO_4$

A different synthesis was attempted to try to generate a isoquinoline framework similar to the lead framework, 3-hydroxy-2-(aminomethyl)pyridine. The Mannich reaction from chapter six proved very useful in generating a library of QMPs. However, no isoquinolinols are commercially available. If 4-isoquinolinol can be made, a Mannich reaction similar to what has been previously discussed could be preformed to generate the desired substituted isoquinolines (Scheme 7.4). A procedure to generate 4-isoquinolinol was therefore investigated.



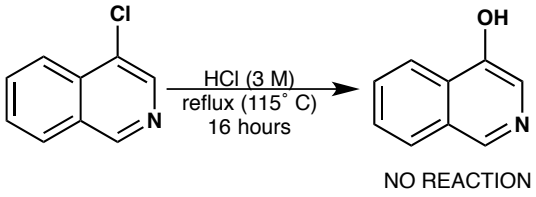
Scheme 7.4: Retrosynthetic analysis of the formation of 4-hydroxy-3-(aminomethyl)isoquinoline from 4-isoquinolinol

A procedure designed by Stacy Fosu from Dr. David Nagib's group at The Ohio State University was utilized in order to place a chlorine group onto the isoquinoline ring at the 4-position.[3] This synthesis from isoquinoline provided high yields of the 4-chloroisoquinoline (Scheme 7.5).



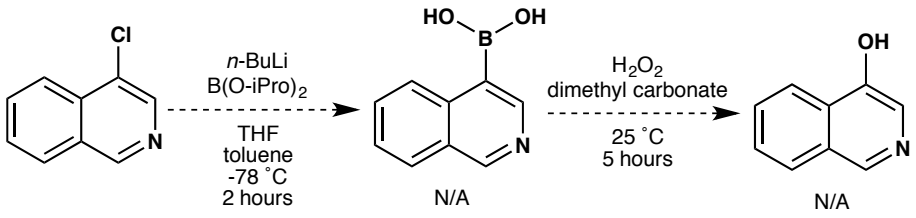
Scheme 7.5: Synthesis of 4-chloroisoquinoline from isoquinoline

With compound **7.3** in hand we next attempted a NAS reaction on the framework, but no substitution was observed (Scheme 7.6).



Scheme 7.6: Attempted synthesis of 4-isoquinolinol from 4-chloroisoquinoline via an NAS reaction

Alternate conditions were researched to generate the isoquinolinol compound. A reaction was found that utilized *n*-BuLi and diisopropyl borate to convert chlorines on aromatic compounds into boronic acids[4]. The boronic acid could then be converted into a hydroxyl group under oxidation conditions with the use of peroxide and dimethyl carbonate (Scheme 7.7)[5].



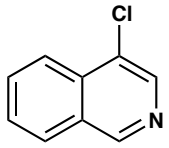
Scheme 7.7: Attempted synthesis of 4-isoquinolinol from 4-chloroisoquinoline

Thus far, the attempts to isolate the boronic acid have failed. Trials are being conducted to determine the correct pH for extraction and isolation of the product. This synthesis is still currently being investigated. If this conversion is successful, it could potentially be used on previous frameworks to convert the chloro groups.

In the future, our hope is a variety of substituted isoquinolines can be generated for testing as a library of QMPs.

7.3 Experimental Data

General Methods The solvents used in these experiments were not purified any further. Unless otherwise noted, all reactions were carried out under standard atmospheric pressure and monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light. Solvents were evaporated under reduced pressure and below 50 ˚C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Starting materials were purchased from reputable suppliers (Sigma Aldrich, Fisher Scientific, Acros Organics, Matrix Scientific Synthonix) and used without further purification. Chromatography was performed on silica gel 60 (40-60 μM), or using Teledyne Isco CombiFlash R_F+UV autocolumn system. ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$). High-resolution mass spectra were recorded on a Bruker MicroTOF II instrument with internal sodium formate as an analyte under electrospray ionization (ESI) conditions.



7.3

4-chloroisoquinoline (7.3) To a solution of dichloroethane (40 mL), iodobenzene diacetate (7.48 g, 23.22 mmol), and isoquinoline (1.82 mL, 15.48 mmol) was added

acetyl chloride (5.51 mL, 77.40 mmol). The reaction mixture was heated to 50 ˚C for 24 h. The reaction was cooled to rt and then neutralized with sat. $NaHCO_3$ (pH = 7). The reaction was extracted with sat. $NaHCO_3$, and then again with sodium dithionite. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude product was purified by chromatography (10:1 hexanes:EtOAc) to yield **7.3** (2.48 g, 98%) as a yellow oil: R_f 0.80 (10:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.16 (d, 1 H, *J* = 1.2 Hz,), 8.59 (d, 1 H, *J* = 1.2 Hz), 8.20 (dd, 1 H, *J* = 1.2, 8.4 Hz), 8.00 (dd, 1 H, *J* = 1.2, 8.4 Hz), 7.83 (td, 1 H, *J* = 1.2, 8.4 Hz), 7.69 (td, 1 H, *J* = 1.2, 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.09, 141.79, 133.58, 131.48, 129.42, 128.49, 128.21, 127.78, 123.34; HRMS (ESI) calcd for (M+Na) C_9H_6ClN: 186.0086, found 186.0069.

7.4 References for Chapter 7

(1) Metcalf, B.; Chuang, C.; Warrington, J.; Paulvannan, K.; Jacobson, M. P.; Hua, L.; Bradley, M.; *World Intellectual Property Organization*. **2013.**

(2) Shi, S.; Wei, X.; Shimizu, Y. Kanai, M.; *J. Am. Chem.* **2012**, *134*, 17019-17022.

(3) Procedure developed from Stacy Fosu from Dr. David Nagib's lab, The Ohio State University, **2017**.

(4) Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, P. S.; Cai, D.; Larson, R. D.; Reider, P. J. *J. Org. Chem.* **2002**, *67*, 5394-5397.

(5) Wagh, R. B.; Nagarkar, J. M. *Tetrahedron Lett.* **2017**, *58*, 4572-4575.

CHAPTER EIGHT

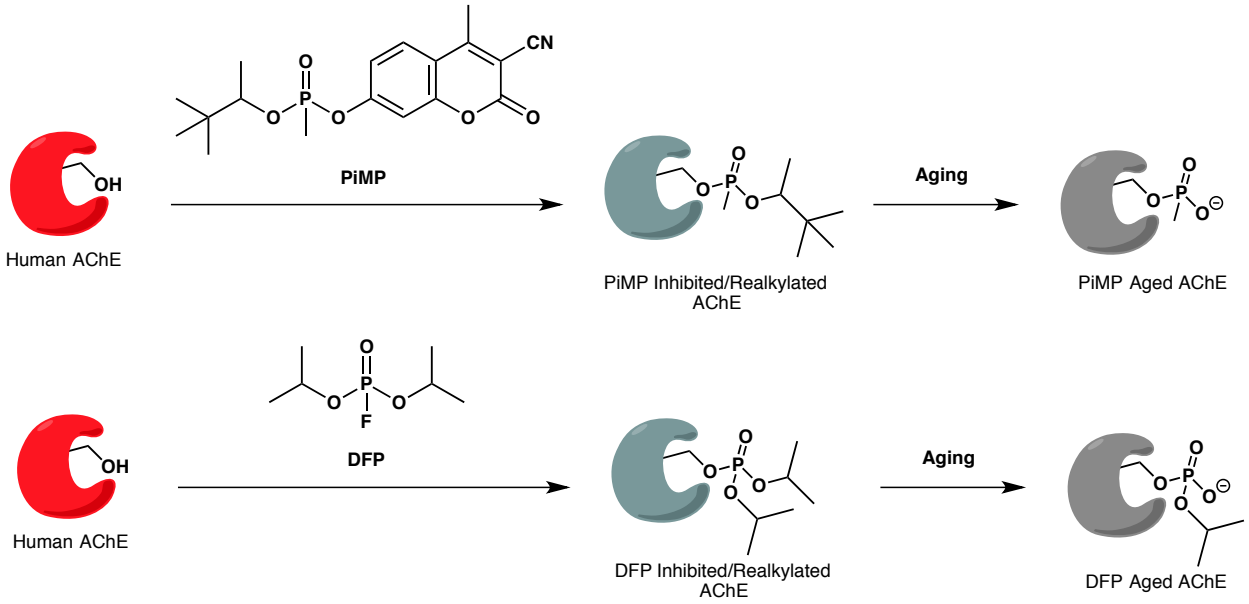
QMP REALKYLATOR SCREENING

8.1 Introduction

The goal in generating libraries of QMP compounds is to screen their respective ability to realklyate and reactivate the aged phosphylated serine residue. This is done in order to determine which framework should be modified and tuned to have the correct reactivity to be our drug of choice when we move to *in vivo* testing.

The QMP assays were first performed with electric eel acetylcholinesterase due to its low cost, commercial availability, and its general similarity in structure to human AChE. Recently, we have obtained a quality source of human AChE and have used that in the assay to better understand the activity these compounds might have on aged human AChE *in vitro*. Human AChE is first incubated with an organophosphorus nerve agent in order to inhibit the enzyme. The inhibited AChE complex is then allowed the appropriate amount of time for aging. The complex is then treated with the most common oxime on the market, 2-PAM, in order to effectively reactivate any inhibited, but unaged, enzyme. The sample is then screened by an Ellman's assay for residual AChE activity. If any activity is present, the inhibition and aging process is repeated. This procedure ensures the complete aging of the enzyme prior to screening any QMP alkylators and creates a quality negative control sample for comparison. For the purpose of these screens, either diisopropyl fluorophosphates (DFP) or PiMP was used to age acetylcholinesterase (Scheme 8.1). These compounds are chosen due to their decreased volatility and therefore increased safety in a lab setting, since they are solids rather than liquids at room temperature. The aging products of these compounds are

analogues of other common nerve agents like soman and VX. PiMP more closely resembles authentic chemical nerve agents in the broad class of organophosphorus compounds, while DFP is a pesticide.

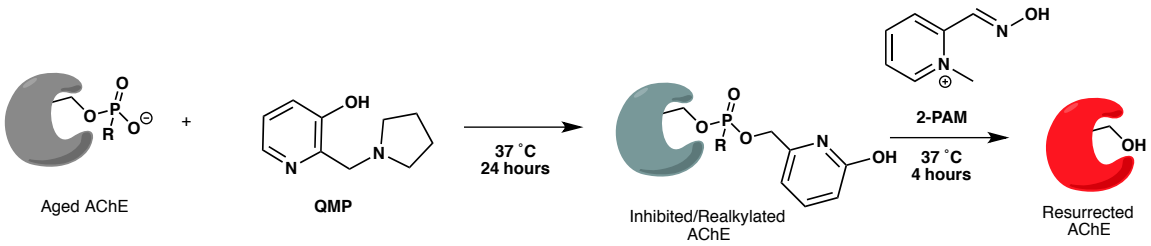


Scheme 8.1: Inhibition and aging of human AChE with PiMP and DFP respectively

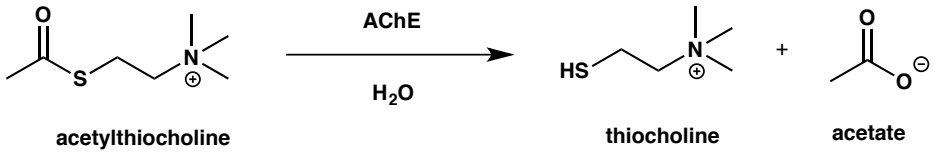
Once the aged AChE sample is obtained, it is incubated with QMP alkylators in the presence of 2-PAM (10 micromolar) under near physiological conditions (37 ˚C, phosphate buffer pH 7.5) for 24 hours (Scheme 8.2). Following this time period, 2-PAM is added to the system. The purpose of adding 2-PAM is to reactivate the realkylated enzyme, thereby regenerating the active, native enzyme. The enzyme activity is then evaluated by Ellman's assay.

The activity of AChE following realkylation by the QMPs and the subsequent reactivation with 2-PAM is monitored by a standard assay known as Ellman's assay.

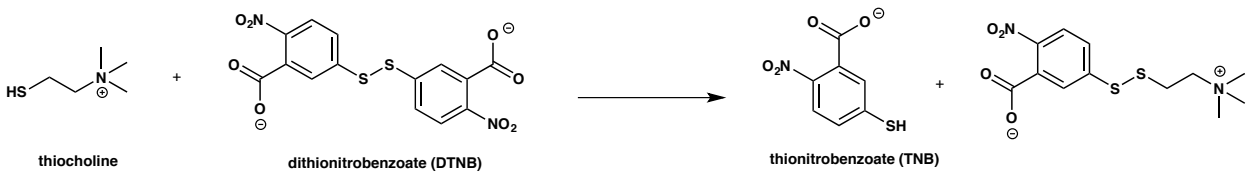
Acetylthiocholine is a substrate for AChE, which is subsequentyl hydrolyzed into thiocholine and acetate by the enzyme (Scheme 8.3). This reaction is analogous to the standard hydrolysis of acetylcholine into choline and acetate. In Ellman's assay, this reaction is performed in the presence of 5,5'-dithiobis(2-nitrobenzoic acid), also known as DTNB. The free thiol in thiocholine reacts with DTNB, cleaving its disulfide bond, to produce yellow 2-thio-5-nitrobenzoate (TNB), which has a strong absorption at 412 nm (Scheme 8.4)[1]. The concentration of TNB can therefore be monitored via ultraviolet-visible spectroscopy by studying the absorbance at 412 nm. This absorbance can be used to measure the amount of thiocholine in solution, and therefore the activity of AChE. For there to be any thiocholine in solution and therefore TNB, there must be active AChE in solution to produce the thiocholine. If any AChE is active, then the respective QMP compound must have managed to resurrect the enzyme. The concentration of thiocholine is dependent on the amount of active AChE there is in solution, which corresponds to how well the QMP resurrected the enzyme over the 24 hour period.



Scheme 8.2: Example protocol for realkylation and reactivation of aged human AChE



Scheme 8.3: Hydrolysis of acetylthiocholine by AChE into thiocholine and acetate



Scheme 8.4: Reaction of thiocholine and DTNB to form TNB, which absorbs at 412 nm

This chapter will discuss the results of the various prior mentioned QMPs in resurrection studies with the aged enzyme.

8.2 Results, Discussion, and Conclusions

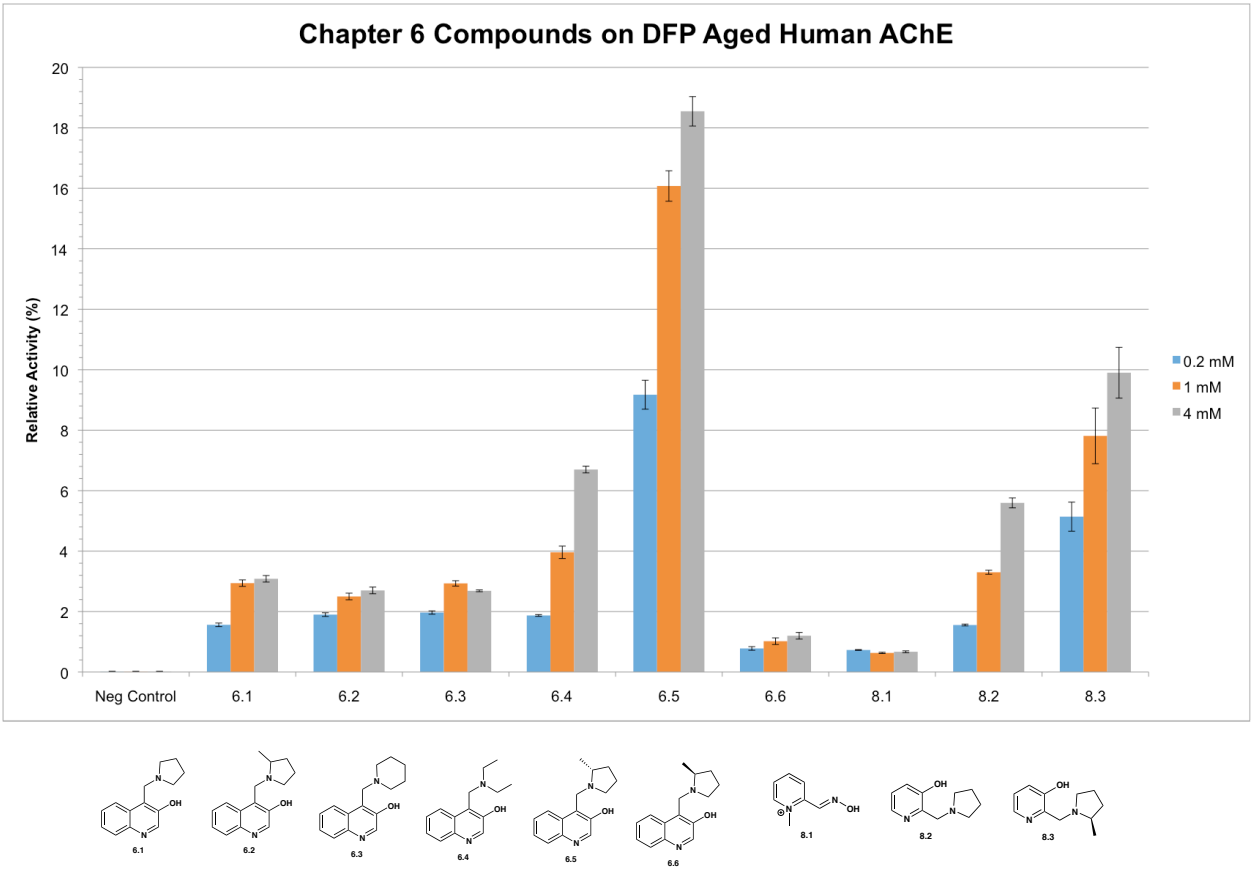


Figure 8.1: Screening results of compounds **6.1-6.6** on DFP-aged Human AChE

Compounds **6.1-6.6**, described in chapter 6, were the first compounds to be screened in the Ellman's assay (Figure 8.1). Each screen was performed using a negative control for a baseline. Compounds **8.1-8.3** screened as well for reference. **8.1**, or 2-PAM, was used to see what the baseline is with just the reactivator and no realkylator. Compounds **8.2** and **8.3** were used to compare the quinolines to the two current lead compounds based off the 3-hydroxy-2-(aminomethyl)pyridine framework.

The quinoline (**6.1**) with a similar pyrrolidine leaving group to pyridine **8.2**, showed similar activity. Both compounds were above the levels for 2-PAM, so it can be said that both compounds show the ability to resurrect, though the levels are quite low, being around or under 5%. At 4 mM, **8.2** performs twice as well as **6.1**. For the (*R*)-2-methylpyrrolidine leaving group, the quinoline (**6.5**) and pyridine (**8.3**) frameworks showed resurrection abilities. The quinoline version however was almost twice as efficient at resurrection in comparison to the pyridine QMPs. It is important to note that the *S* enantiomer **6.6** showed baseline activity, and the racemic **6.2** was worse than *R*. There seems to be something significant about not just the methyl group on the pyrrolidine leaving group, but also the *R* stereochemistry in that position. This group must be having favorable binding interactions with side chains in the active site of AChE. This also has us questioning the mechanism of the formation of a QM, as the leaving group seems to have a very significant impact on the ability of these compounds to resurrect AChE, so the leaving group might be playing a part, since if it was a QM there would be no leaving group on the molecule and therefore it would have no impact. Compounds **6.3** and **6.4**, with piperidine and diethyl amine respectively, also showed the ability to resurrect the enzyme, and have similar rates to the pyrrolidine form.

However, all performed significantly below **6.5**. It seems the key framework revolves around the (*R*)-2-methylpyrrolidine leaving group, and as the quinoline and pyridine have similar relative activity with pyrrolidine, it is hard to ascertain which is superior at this time. On the basis of the (*R*)-2-methylpyrrolidine versions, it seems that the quinoline framework is superior to the pyridine framework for DFP-aged AChE, as it has the highest relative activity after being treated with **6.5** in comparison to any QMP made in our laboratory. It is important to note that reactivity is improved at higher concentrations. Further studies and modifications would need to be made before these compounds could be used for human treatment.

While the quinoline showed resurrection ability with the AChE aged with pesticide organophosphorus compound DFP, it did not show the same results when used on AChE aged with nerve agent PiMP (Figure 8.2). While the pyridine derivatives **8.2** and **8.3** showed promising results with PiMP as well as DFP, **6.5**, which had the most success with DFP, was only slightly above baseline when used on PiMP-aged AChE. Compound **8.2** performed better on PiMP-aged AChE than on DFP-aged. This leads us to believe that different resurrection agents will work better on certain aged AChE forms over others, and multiple drugs would need to be synthesized to combat the various forms of aged enzyme that can come from the available organophosphorus agents. All of the other quinolines were near or at baseline, showing similar activity levels as 2-PAM.

For the following studies on the quinoline compounds, they were solely tested on DFP-aged AChE to see if they would have improved performance over **6.5**. They were

all tested at both pH 7.5 and pH 9 at a concentration of 1 mM. For these comparisons, only the negative control and **6.5** were used as references.

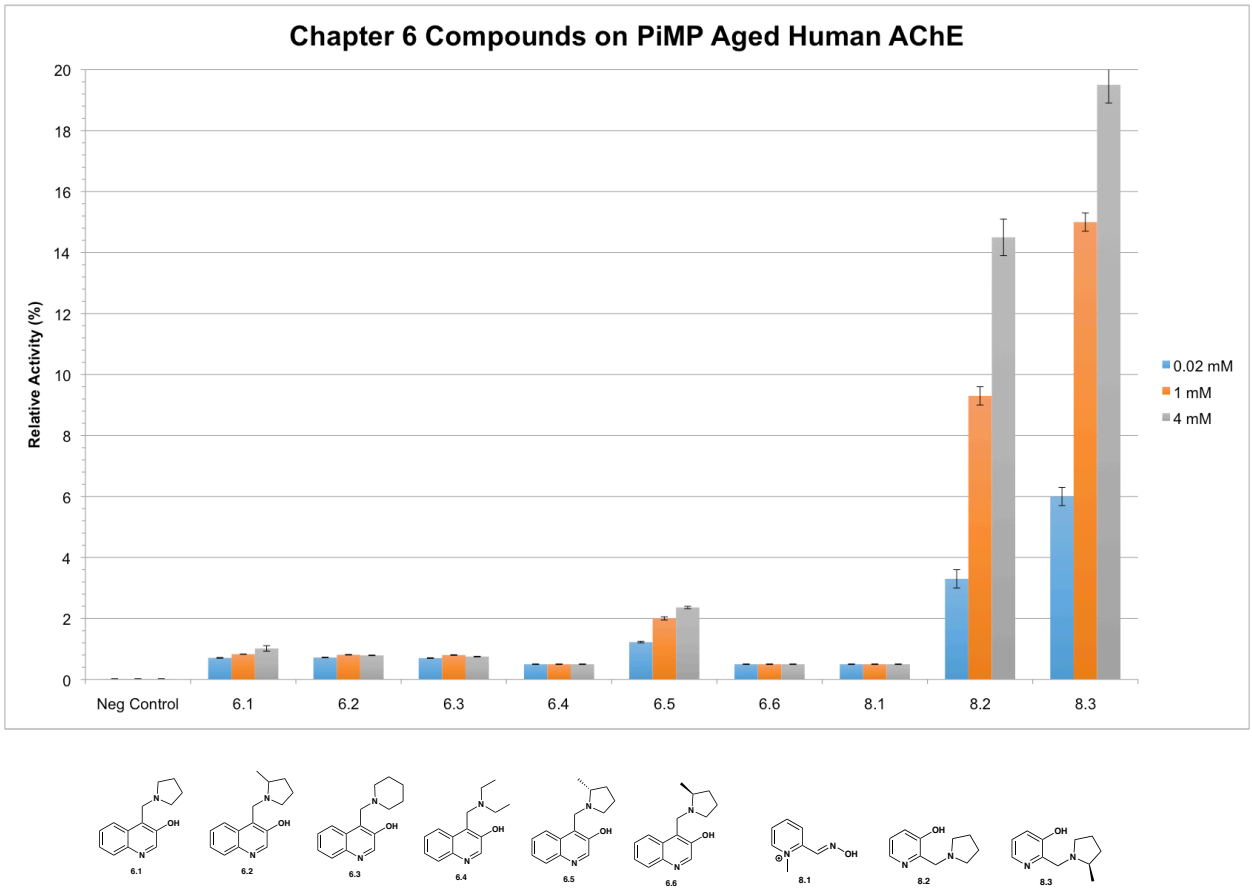


Figure 8.2: Screening results of compounds **6.1-6.6** on PiMP-aged Human AChE

Compounds **2.5-2.8**, **3.7-3.10**, **4.2-4.5**, and **5.2-2.5** were screened as shown in Figures 8.3-8.6. All of the compounds were found to be baseline. Compound **6.5** performed significantly better than the other quinoline QMP frameworks. This compound proves to be special in its abilities to resurrect aged AChE. It is important to note that **6.5** performed better at pH 9 over pH 7.5. As the body's physiological pH is not 9, in order to get better activity with this compound, other substituents should be placed onto

the ring to try to obtain the favorable protonation state that it currently has at pH 9 and pH 7.5 instead. It is also important to note that for these screens, **6.5** performed significantly worse at 1 mM than it did in the prior screen, so these results should be replicated to determine which one has the correct value for the percent activity under these screening conditions. The results can still allow us to compare the various QMPs.

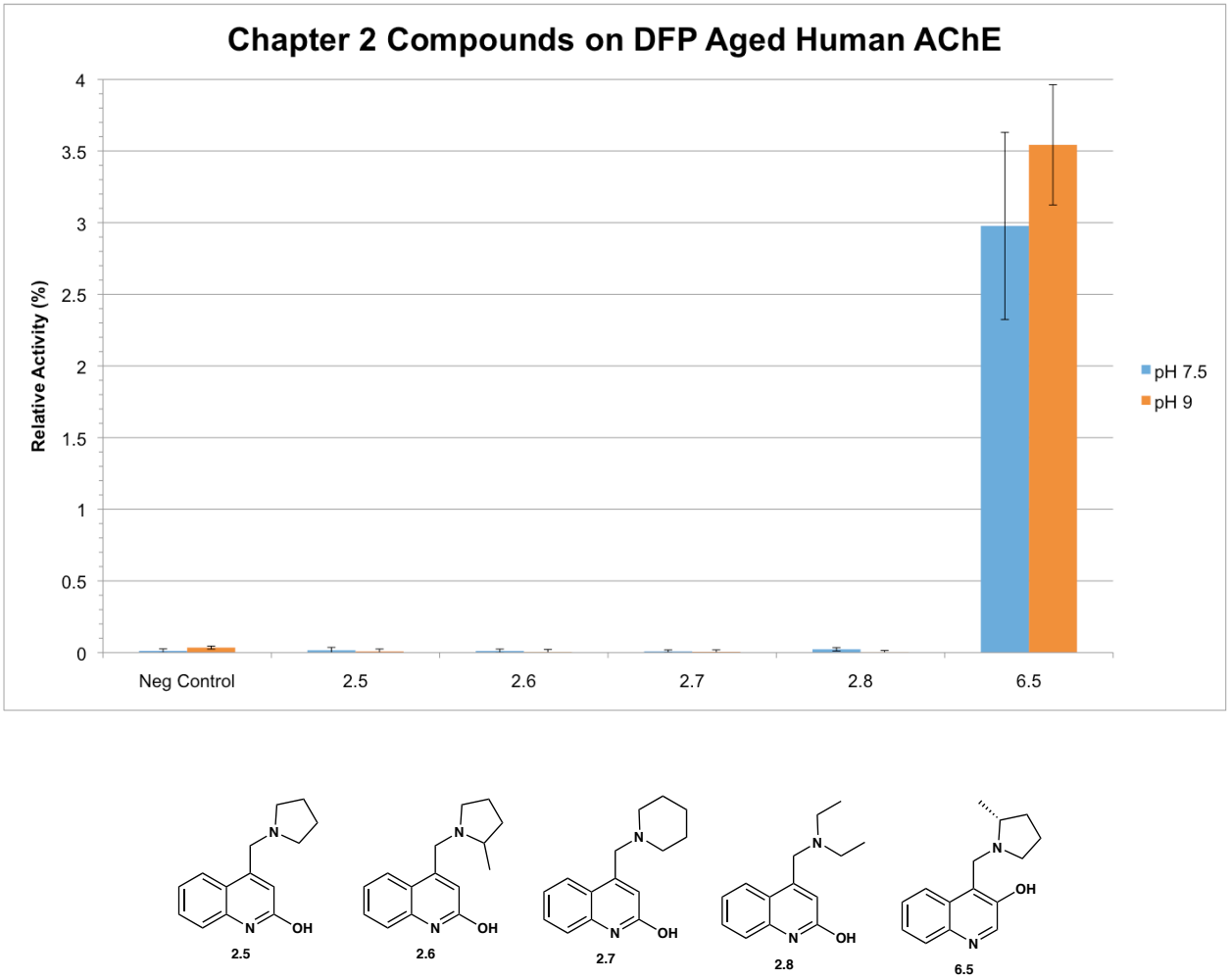


Figure 8.3: Screening results of compounds **2.5-2.8** on DFP-aged Human AChE

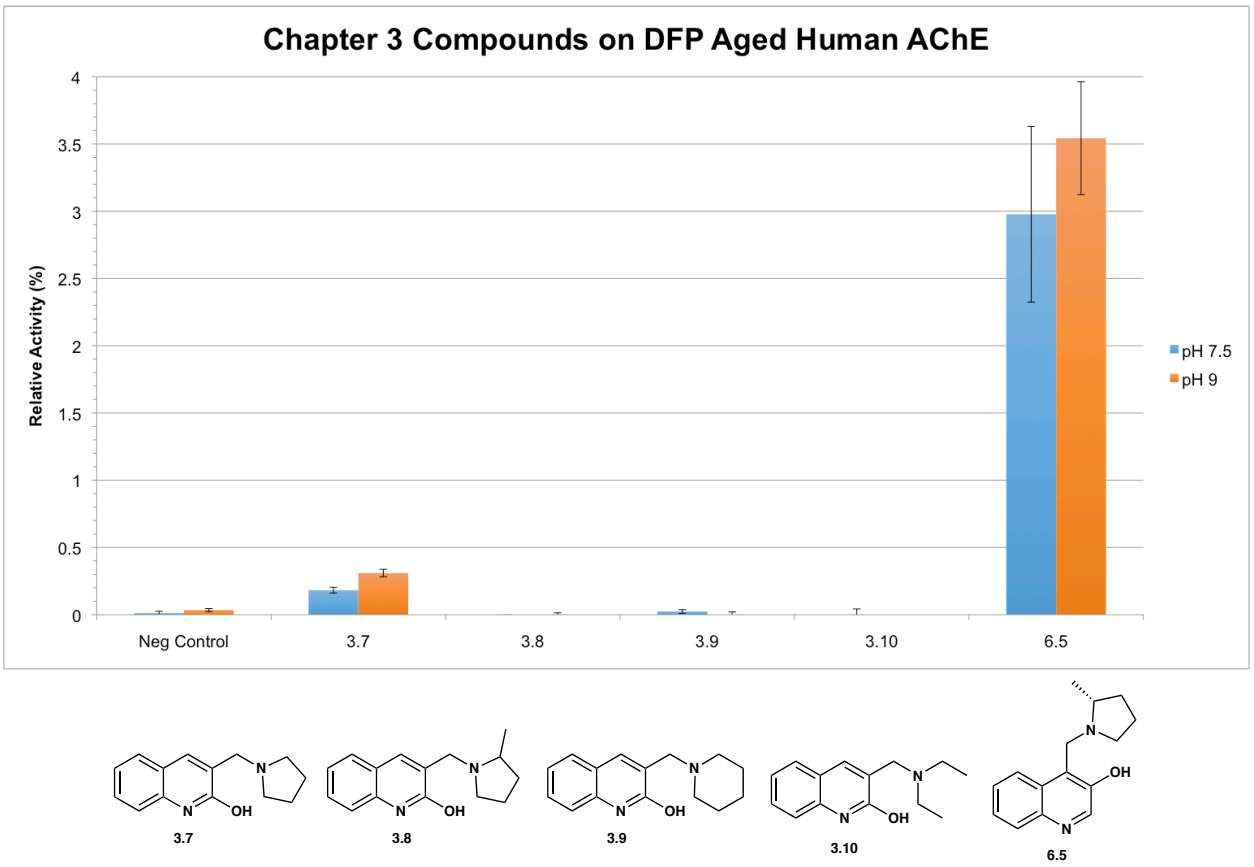


Figure 8.4: Screening results of compounds **3.7-3.10** on DFP-aged Human AChE

The only compound with any resurrection activity besides **6.5** was **3.7**, though since it below 0.5% activity it can be assumed that it is also at baseline and should not be considered successful. The quinolines in the computational modeling have shown to be very good binders the active site of acetylcholinesterase. We hypothesize that they might be binding so well that once they realkylate or reactivate the enzyme, the compounds themselves inhibit the active site and prevent it from performing its catalytic duties. The compounds also showed solubility issues in water.

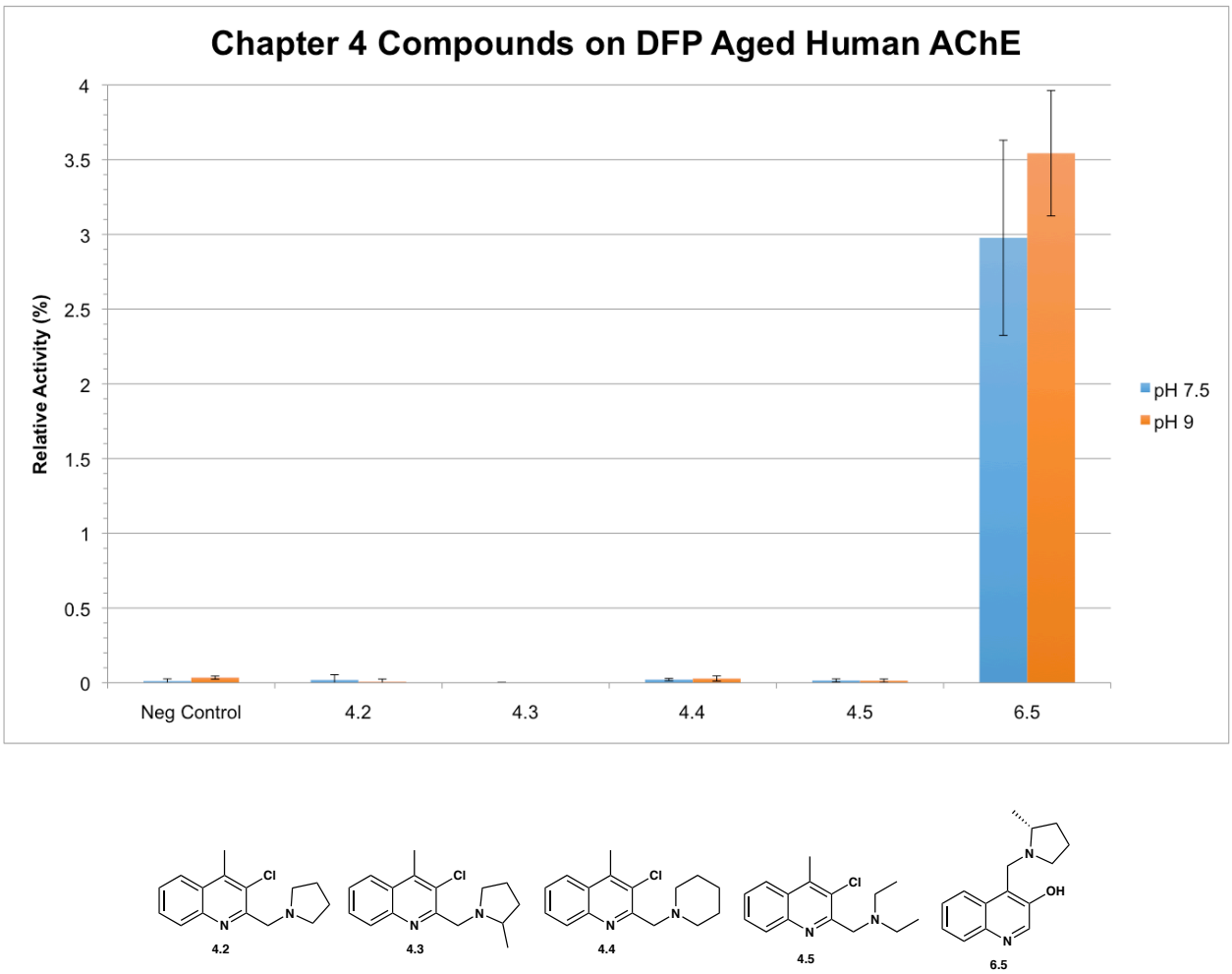


Figure 8.5: Screening results of compounds **4.2-4.5** on DFP-aged Human AChE

As the body is made of water, in order for these compounds to function properly they must be substituted to be more polar so they can dissolve in water and make it to the active site of AChE. Computational modeling also has shown that they should perform significantly better than the pyridine derivatives, so we are currently focused on improving these issues.

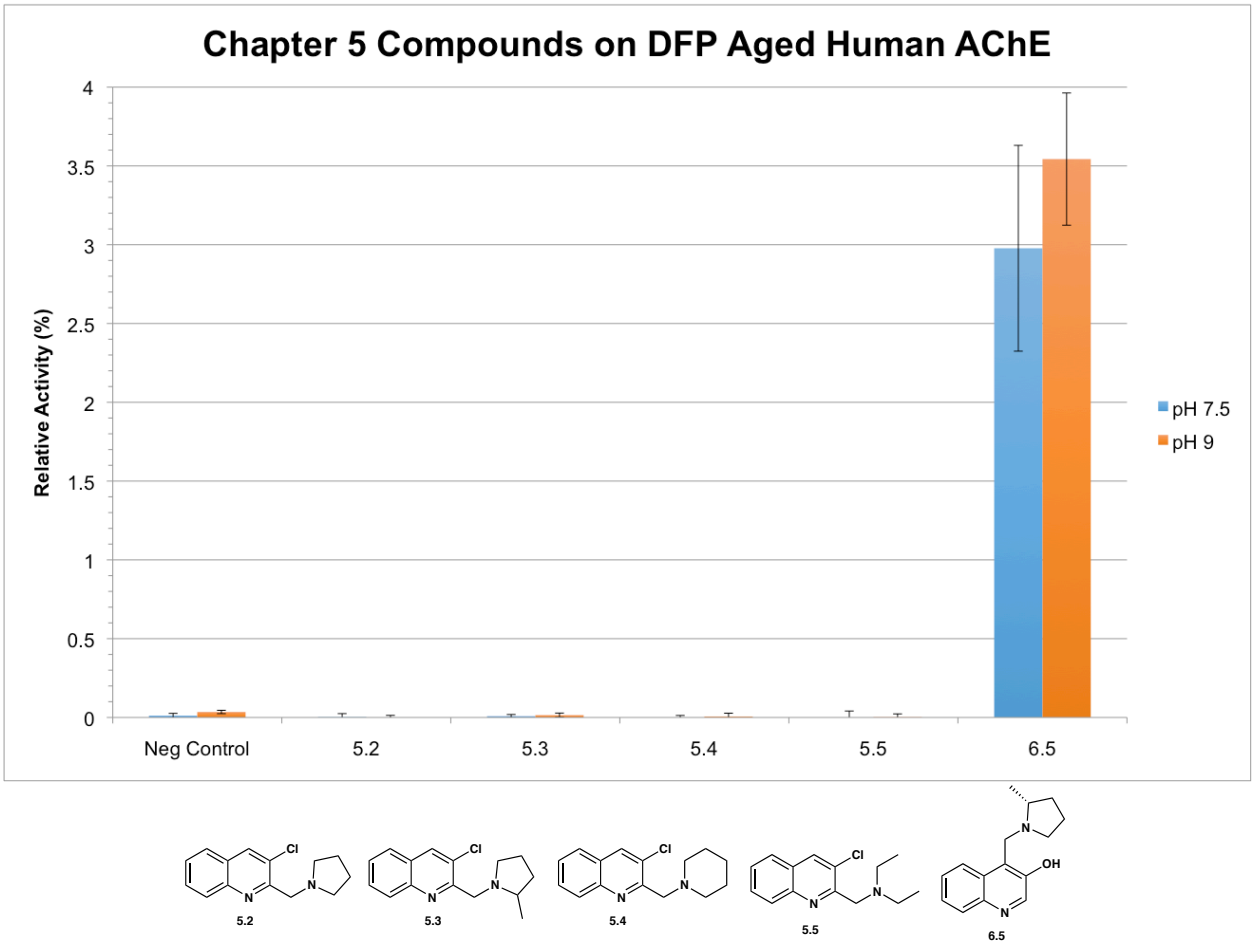


Figure 8.6: Screening results of compounds **5.2-5.5** on DFP-aged Human AChE

Quinoline **6.5** showed the most promise for resurrection of aged AChE. The quinoline QMPs synthesized in this thesis demonstrate a dependence on pH, concentration, and the type of organophosphorus agent used to age the enzyme. A number of new compounds have been synthesized and verified to be resurrectors of the aged form of AChE, and with better activities for exposure to pesticides.

8.3 References for Chapter 8

(1) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70-77.

APPENDIX A

SELECT ^{1}HNMR AND ^{13}CNMR DATA FROM CHAPTER 2

Faculty Group Callam

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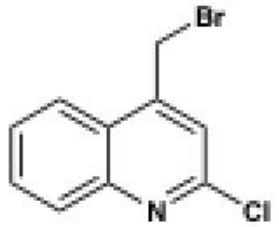
4.795



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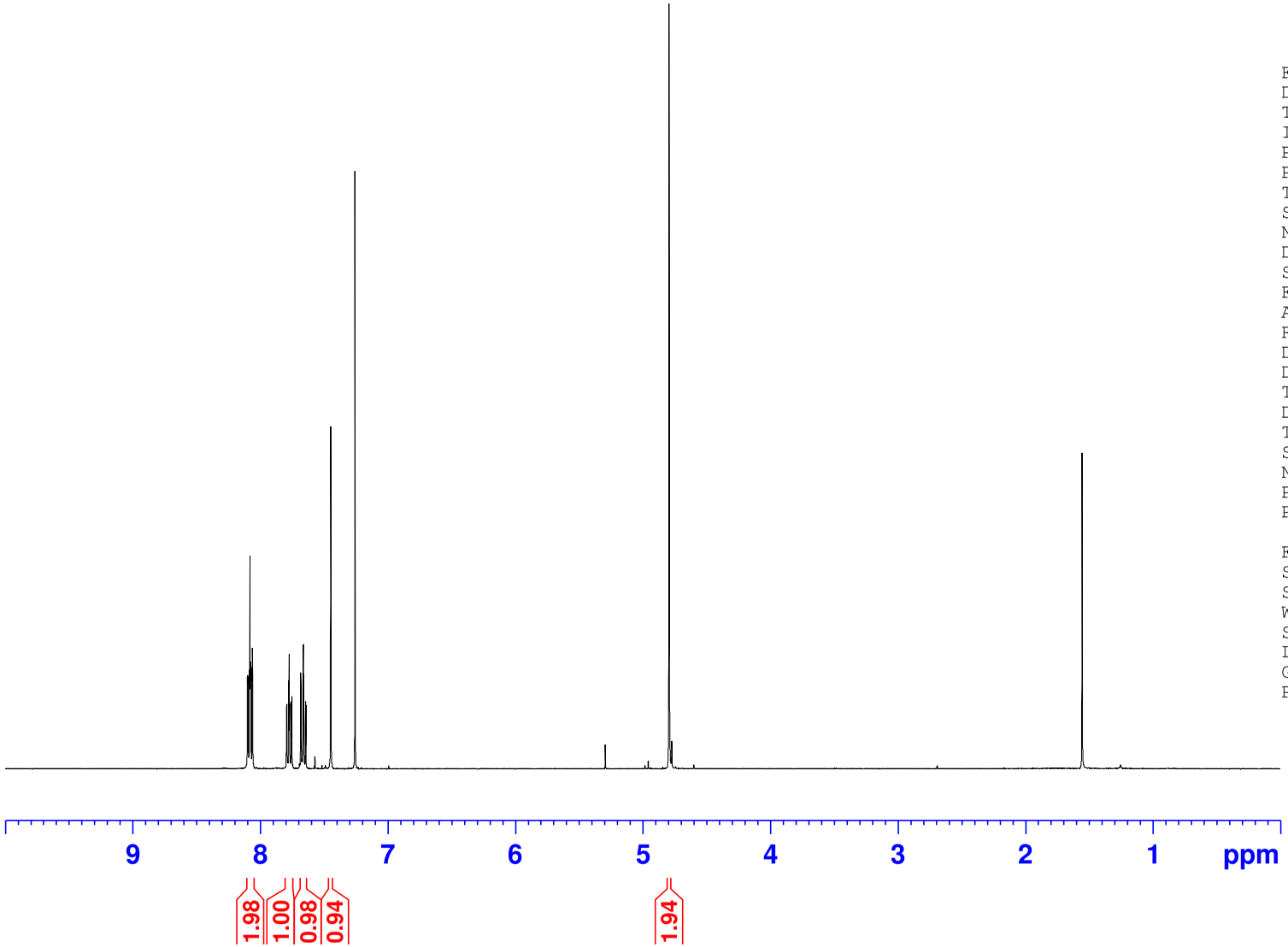

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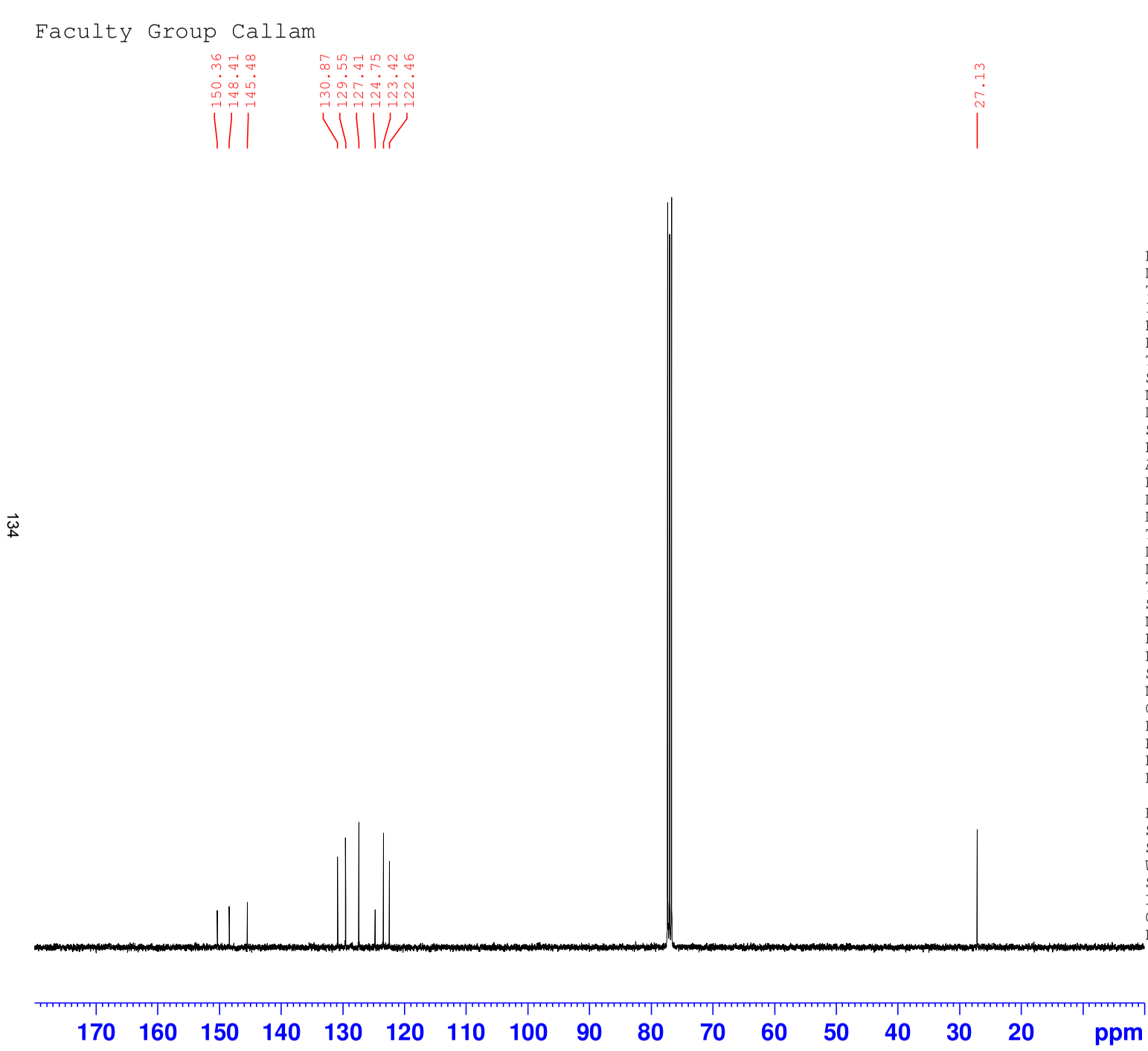
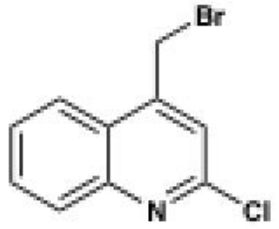
Faculty Group Callam





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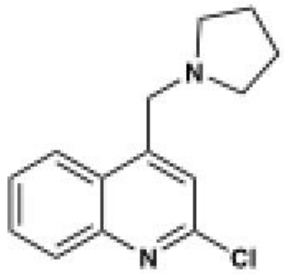

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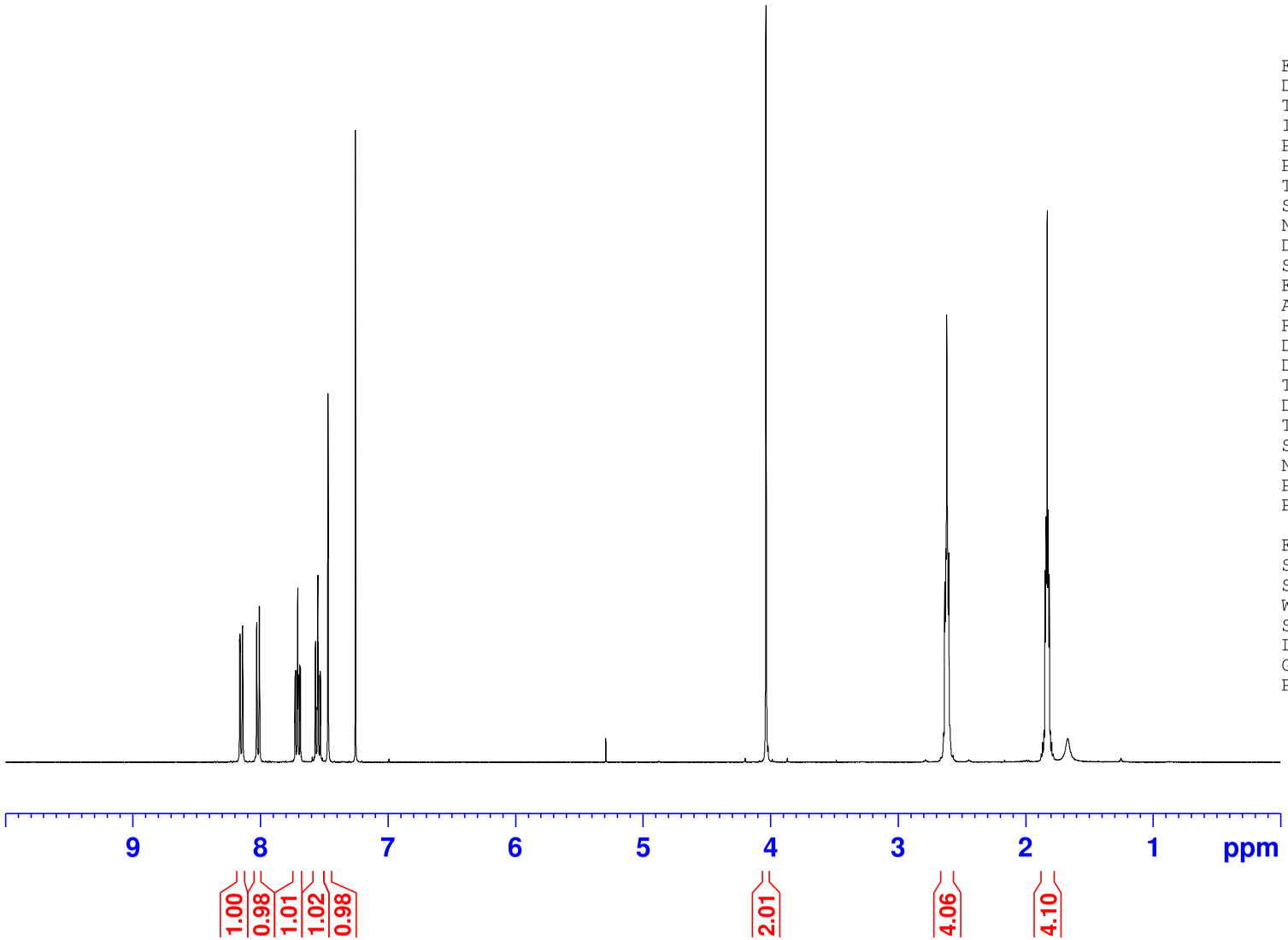

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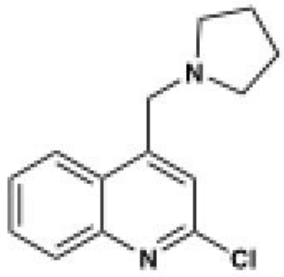

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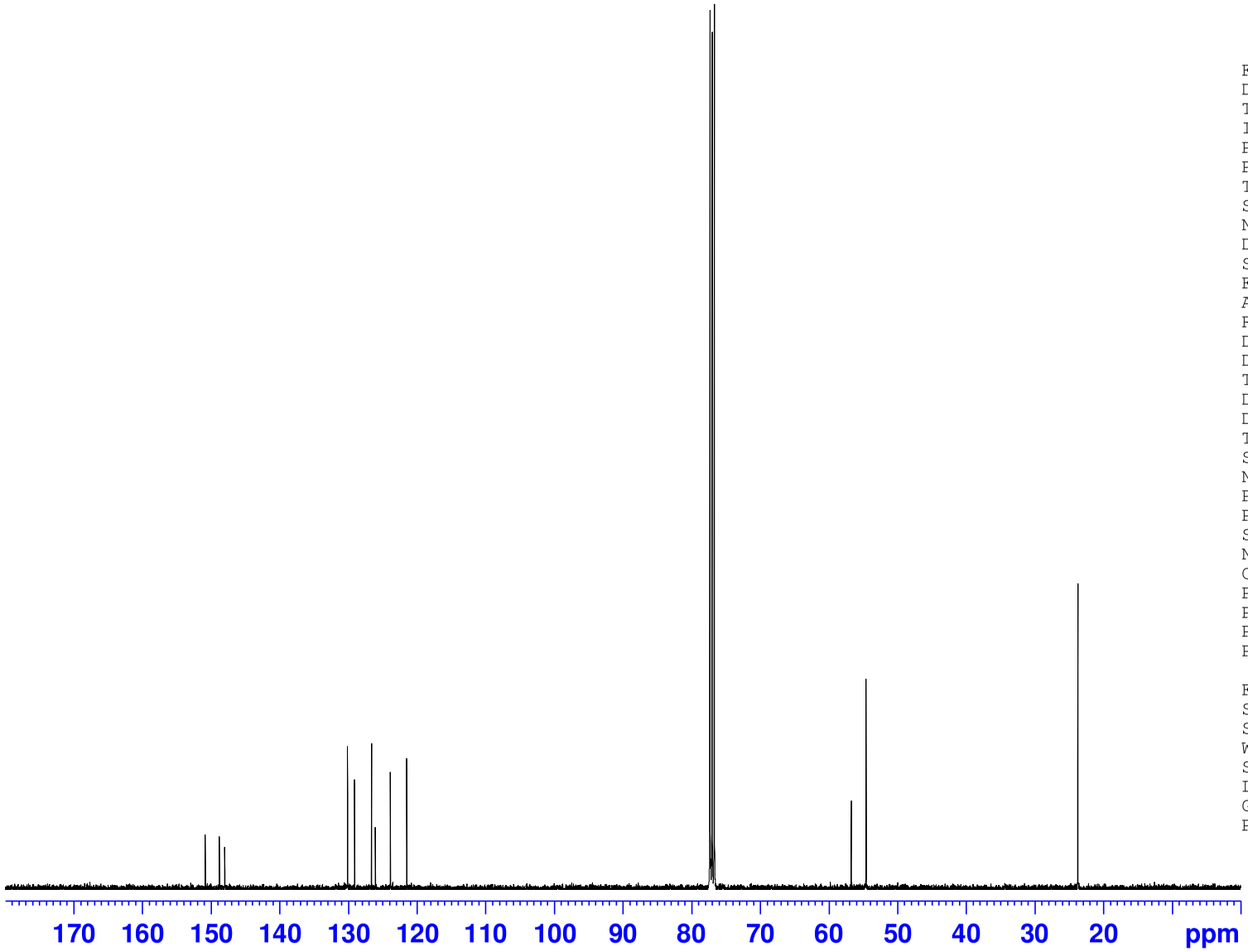

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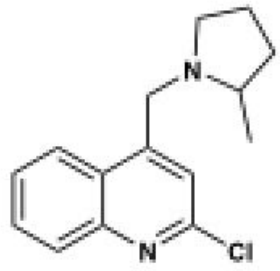
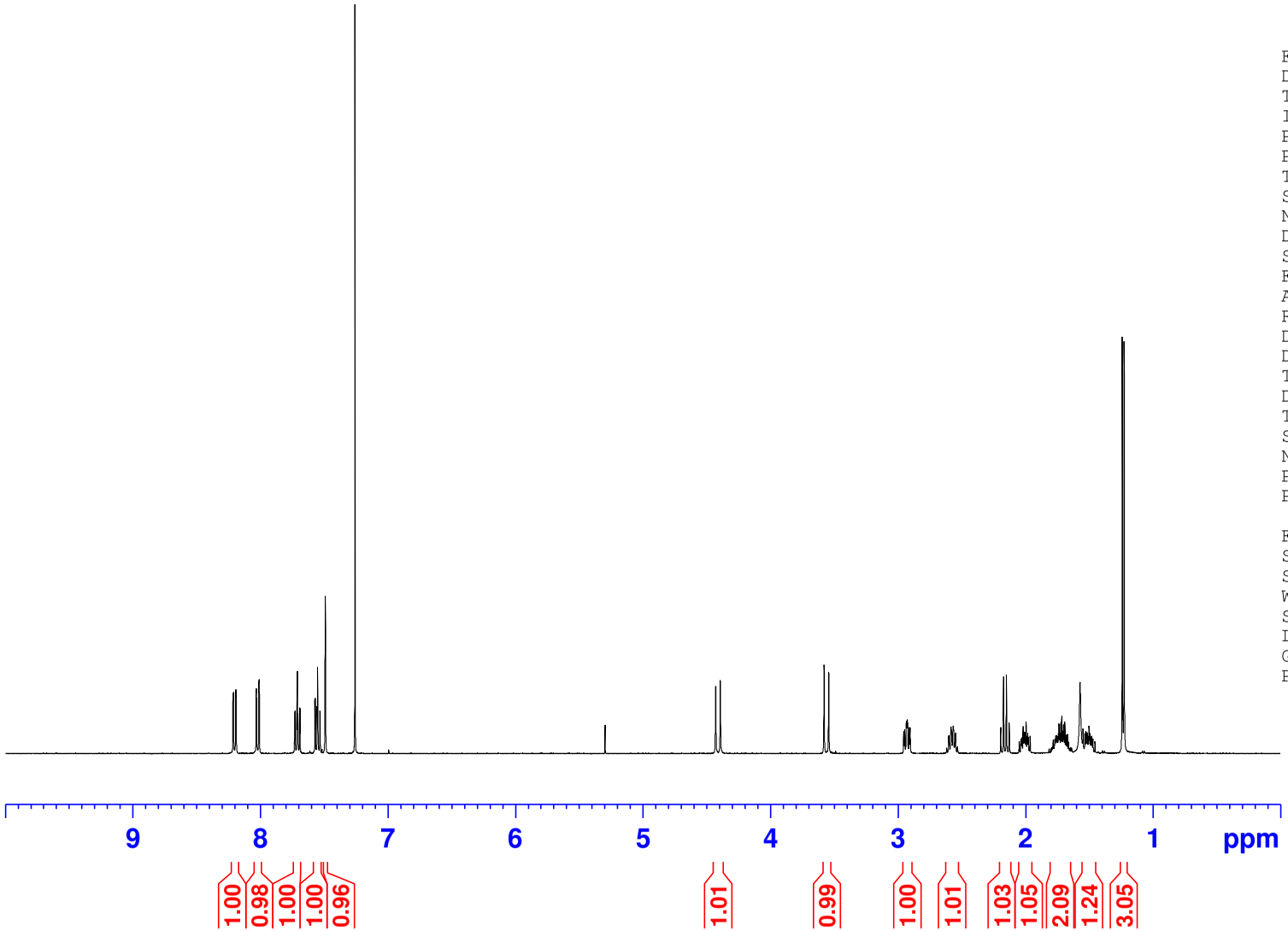
PC	1.40	

Faculty Group Callam

8.215 8.212 8.194 8.191 8.033 8.032 8.013 7.728 7.711 7.694 7.573 7.570 7.552 7.531 7.492 4.431 4.429 4.395 4.393 3.580 3.543 2.955 2.947 2.933 2.927 2.913 2.905 2.584 2.567 2.549 2.194 2.172 2.150 2.128 2.019 2.006 1.995 1.723 1.715 1.707 1.502 1.497 1.241 1.226



2.6



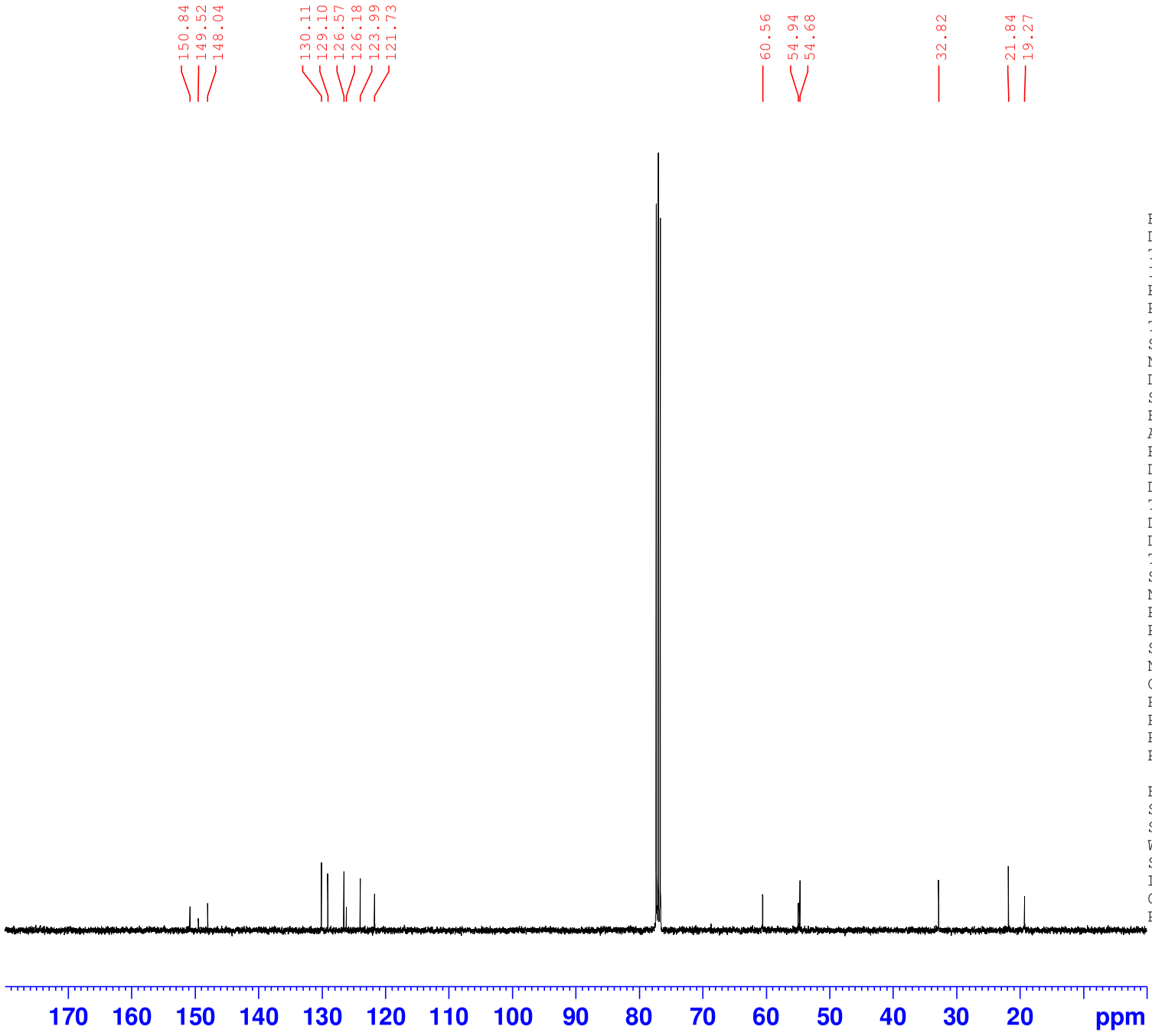
F2 - Acquisition Parameters

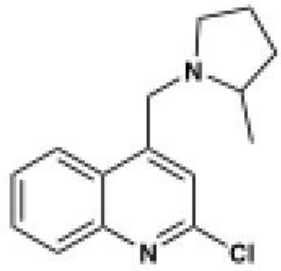
Date_	20180225	
Time	10.59	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700105	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





2.6

```
F2 - Acquisition Parameters
Date_           20180317
Time                5.41 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

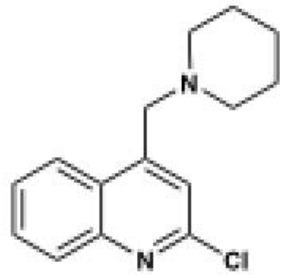
Faculty Group Callam

8.168
8.031
8.029
8.011
8.010
8.008
7.731
7.727
7.713
7.710
7.706
7.692
7.689
7.568
7.565
7.551
7.547
7.544
7.530
7.527
7.473

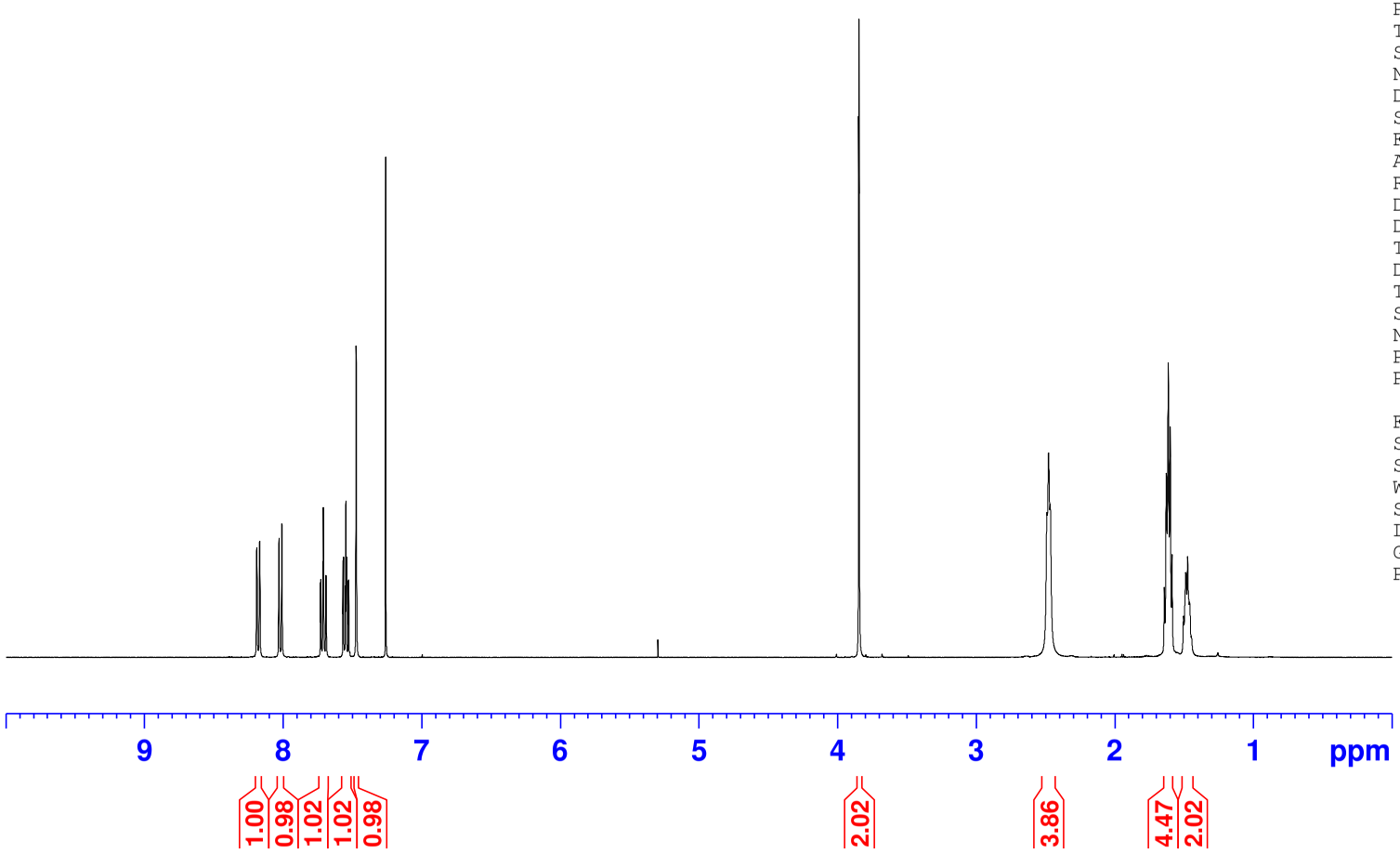
3.844

2.477

1.613
1.474



2.7



F2 - Acquisition Parameters

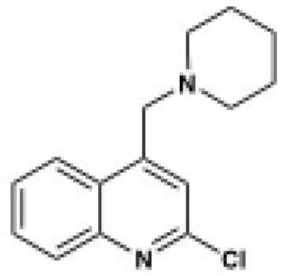
Date_	20180228	
Time	17.49	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

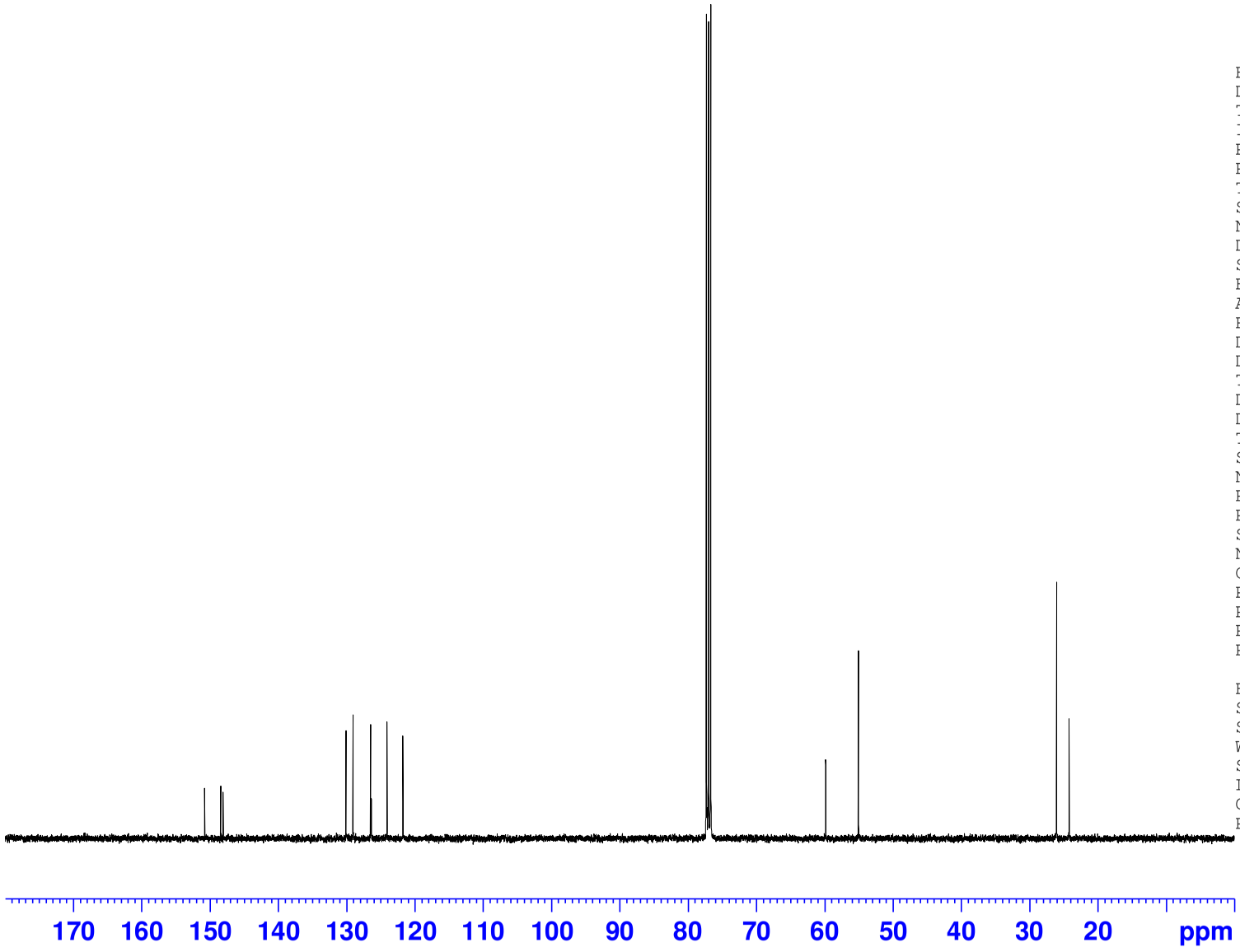
SI	65536	
SF	400.1700101	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

150.81
148.45
148.09
130.11
129.09
126.51
126.40
124.10
121.77
59.85
55.06
26.05
24.21



2.7



F2 - Acquisition Parameters

Date_	20180301	
Time	6.07	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	1820	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

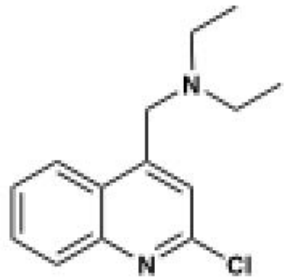
Faculty Group Callam

8.161 8.158 8.033 8.032 8.013 8.012 8.011 7.726 7.723 7.709 7.706 7.702 7.688 7.685 7.562 7.546 7.542 7.539 7.525 7.521

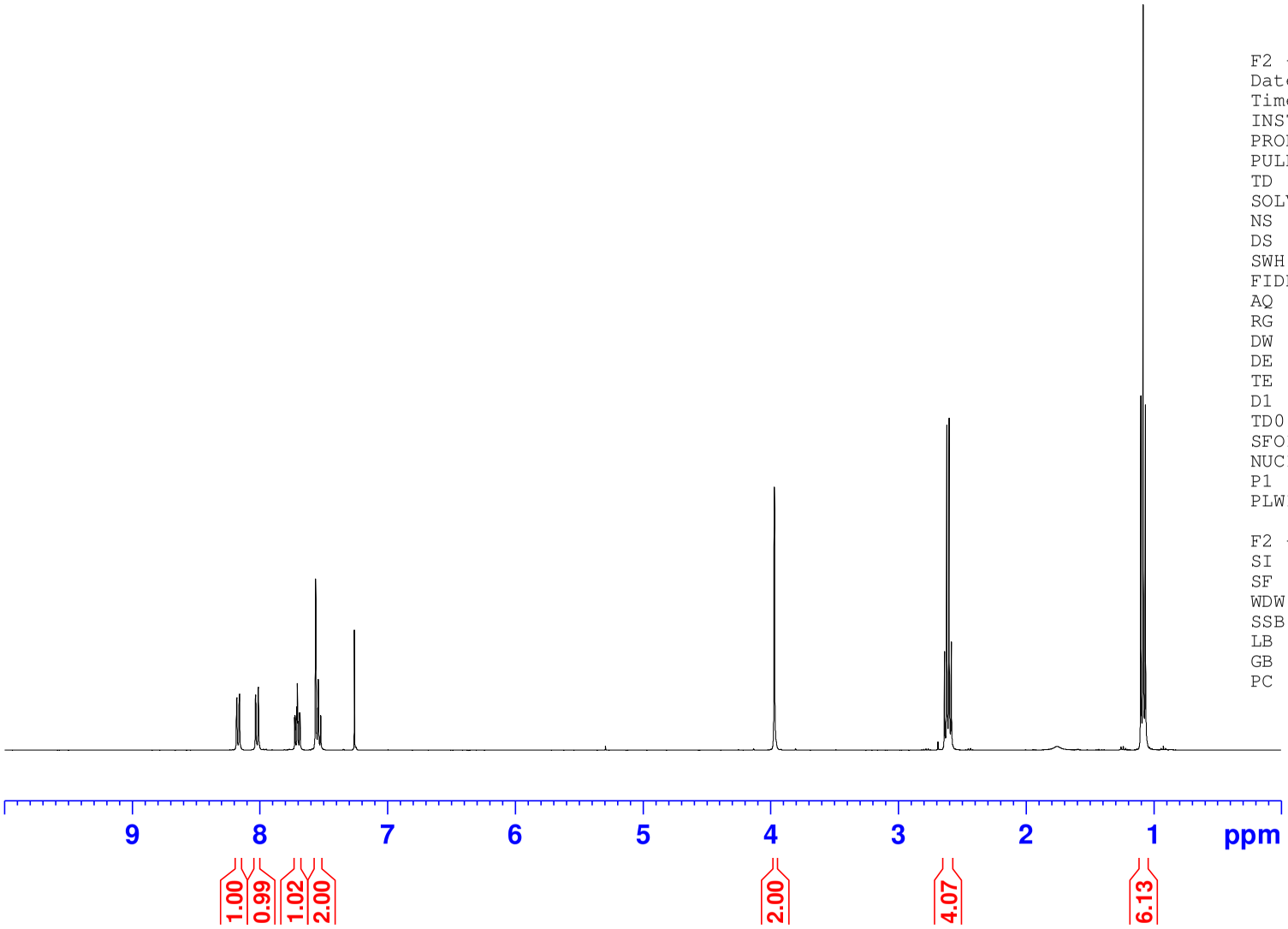
3.971

2.637 2.619 2.602 2.584

1.100 1.082 1.064



2.8



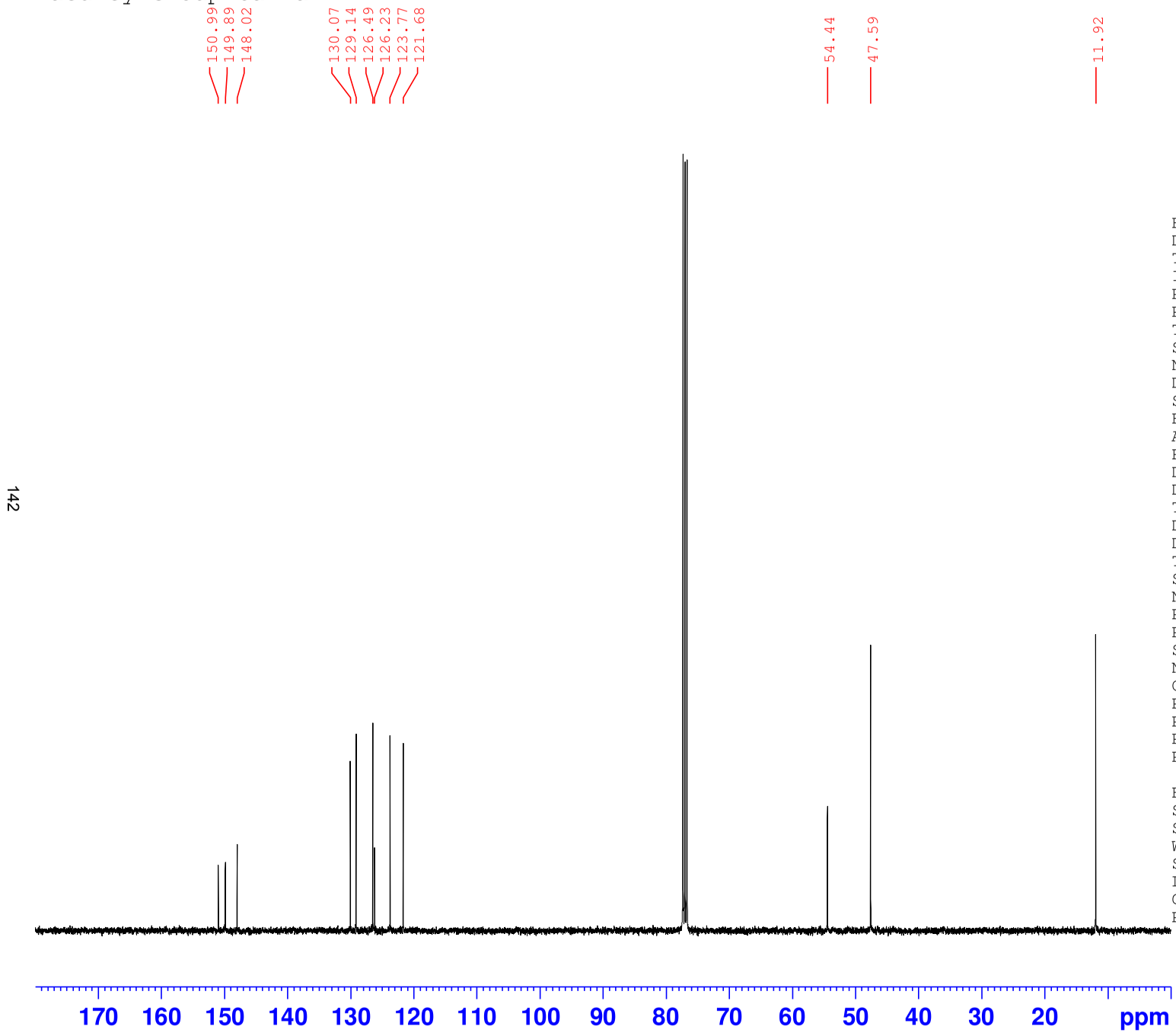
F2 - Acquisition Parameters

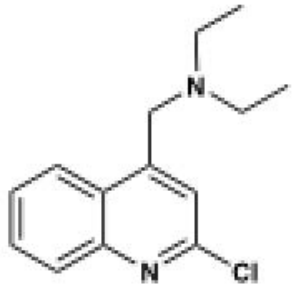
Date_	20180228	
Time	17.54	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	188.13	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700104	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





2.8

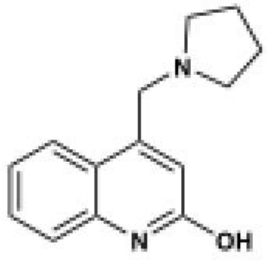
```
F2 - Acquisition Parameters
Date_            20180301
Time                7.10 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ             1.3631488 sec
RG                  2050
DW                20.800 usec
DE                 6.50 usec
TE                300.5 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                 32768
SF           100.6228265 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

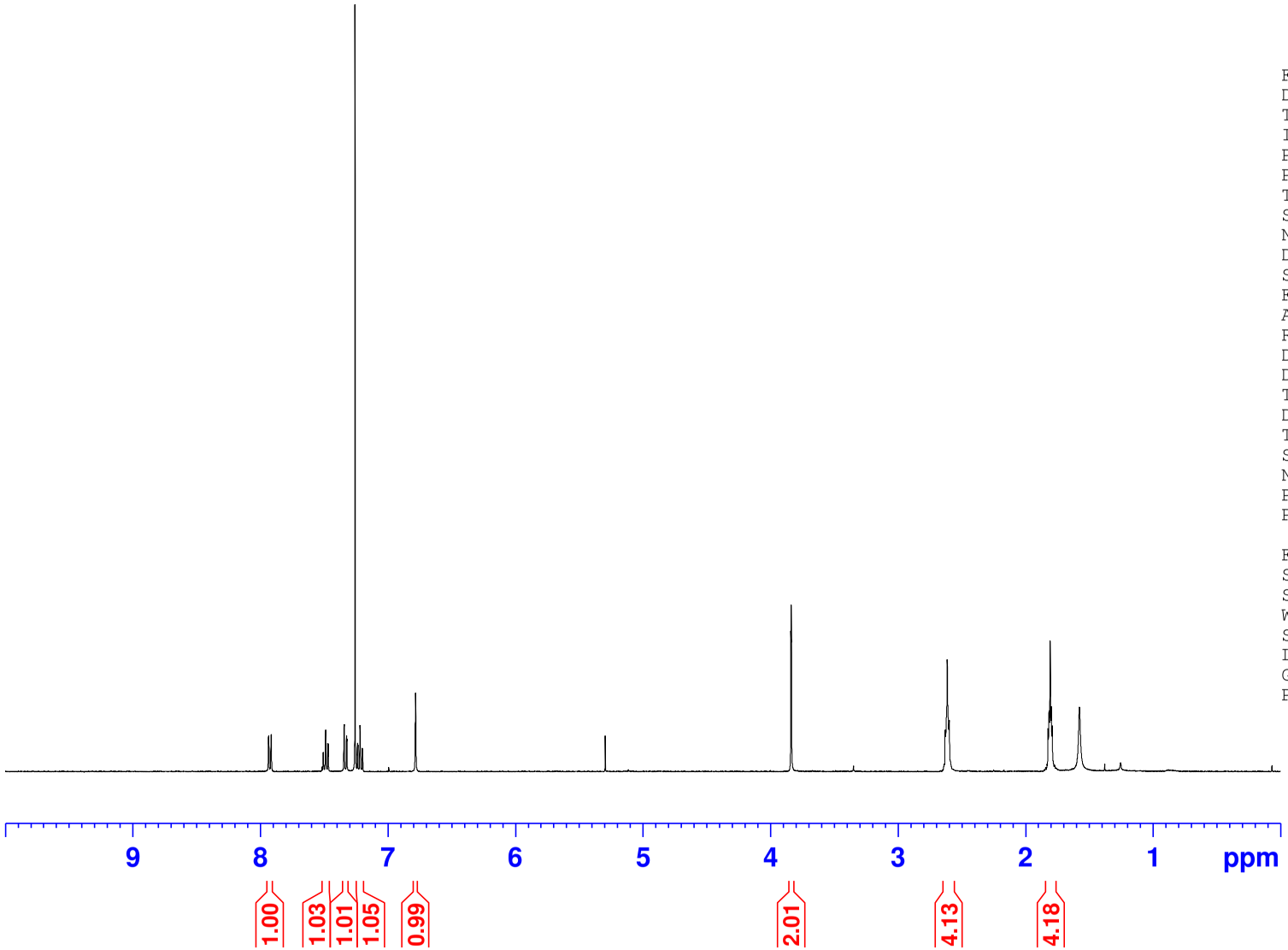
Faculty Group Callam

7.935
7.918
7.915
7.510
7.506
7.492
7.489
7.486
7.471
7.468
7.345
7.343
7.325
7.323
7.239
7.236
7.221
7.219
7.216
7.201
7.198
6.784

3.840
3.837
2.631
2.627
2.620
2.614
2.601
2.597
1.823
1.816
1.807
1.798
1.790
1.578



2.9



F2 - Acquisition Parameters

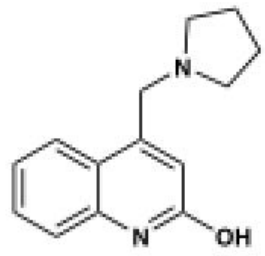
Date_	20180228	
Time	17.59	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

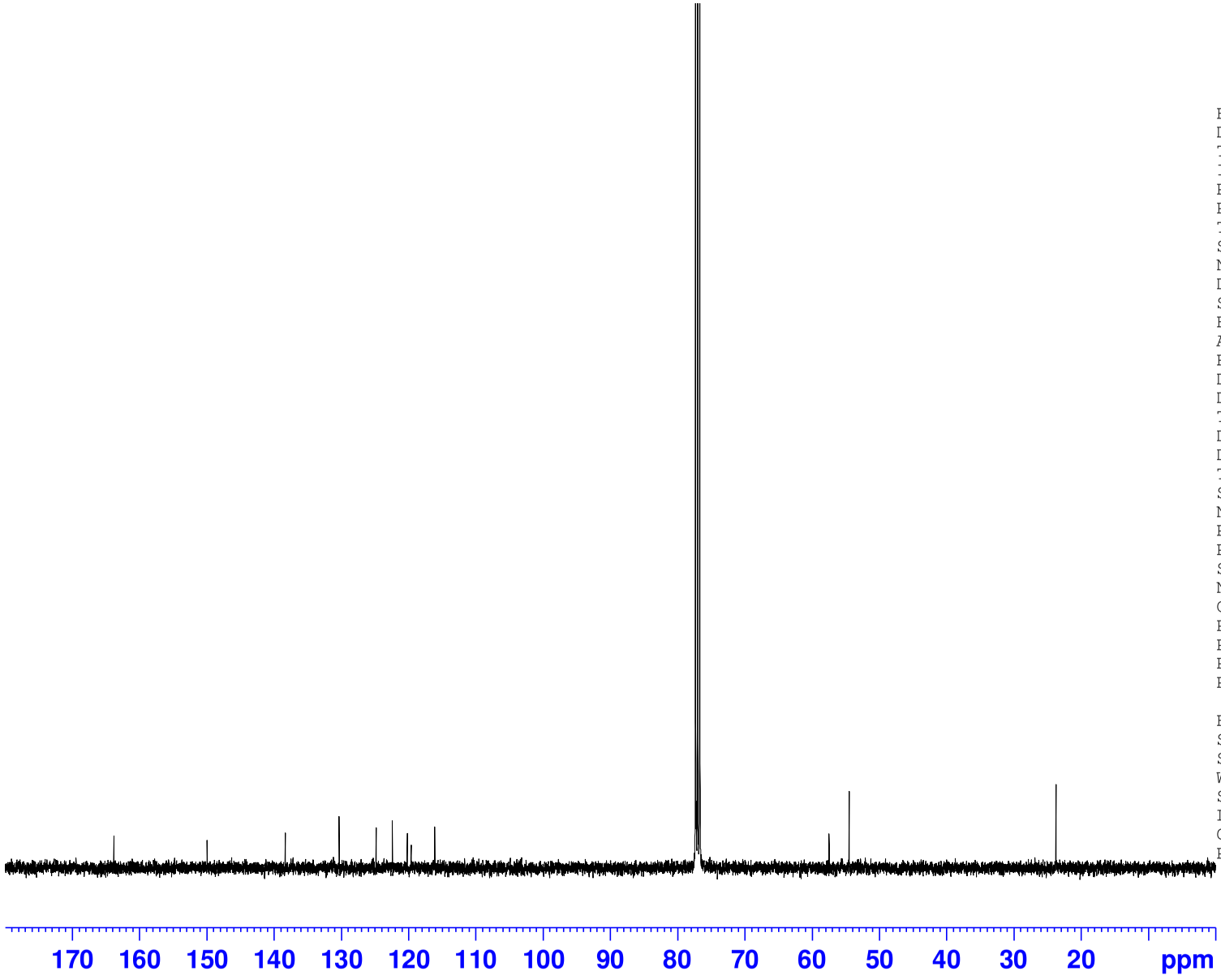
SI	65536	
SF	400.1700108	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

163.84
149.97
138.34
130.35
124.83
122.44
120.21
119.63
116.12
57.47
54.48
23.73



2.9



F2 - Acquisition Parameters

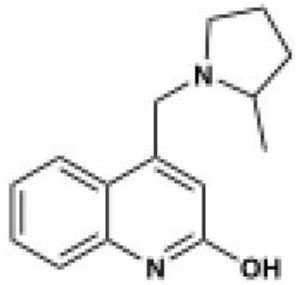
Date_	20180302	
Time	0.35	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

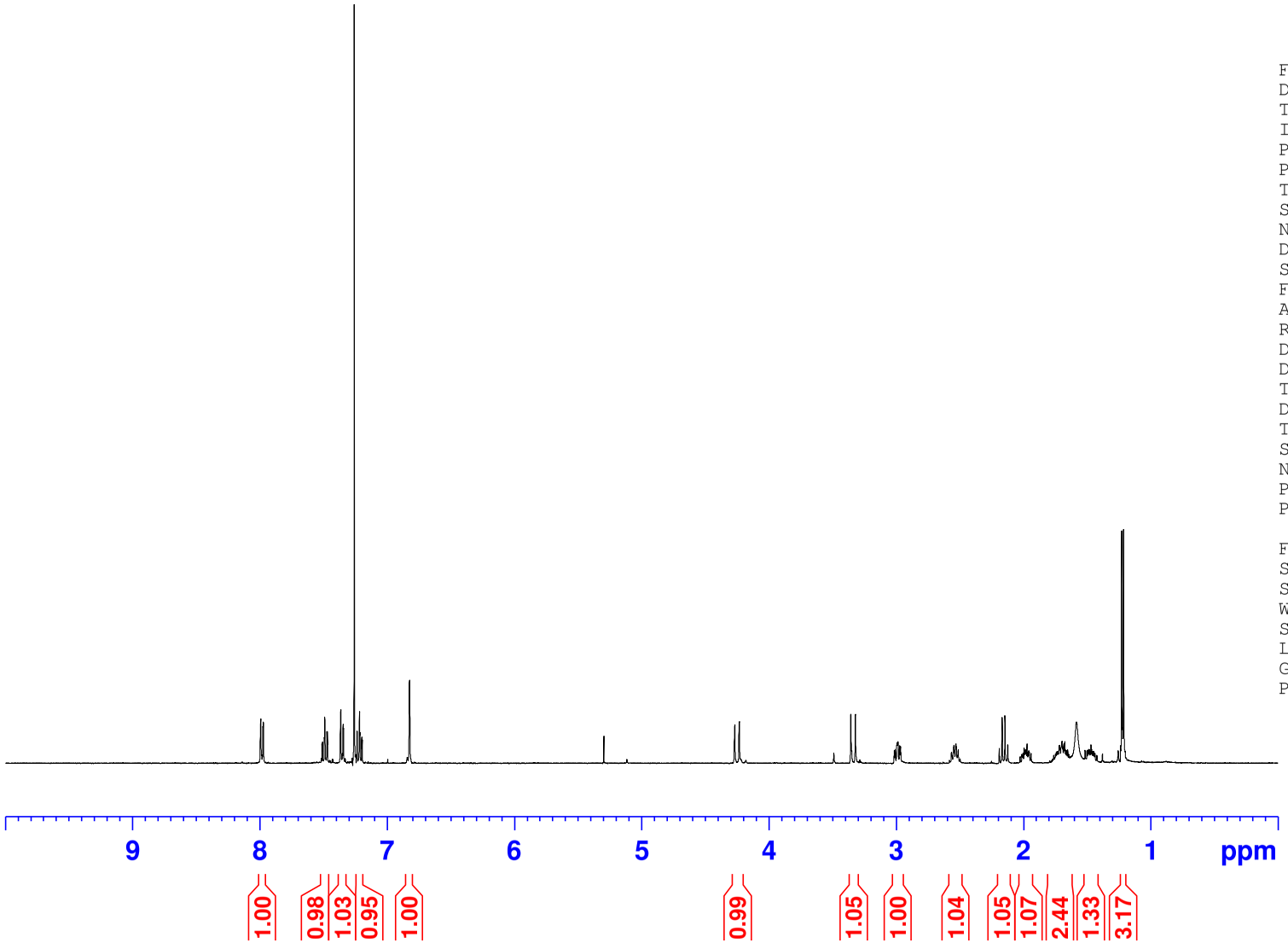
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam

7.996 7.993 7.976 7.973 7.511 7.508 7.493 7.491 7.488 7.473 7.470 7.367 7.365 7.346 7.344 7.238 7.235 7.220 7.218 7.215 7.200 7.197 6.825 4.271 4.268 4.235 4.233 3.356 3.320 2.993 2.986 2.547 2.531 2.168 2.146 2.124 1.972 1.714 1.697 1.682 1.675 1.470 1.229 1.214



2.10



F2 - Acquisition Parameters

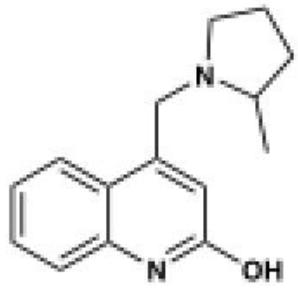
Date_	20180228	
Time	18.05	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700103	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

163.99
150.67
138.36
130.32
124.88
122.39
120.37
119.71
116.17
60.39
55.63
54.56
32.89
21.85
19.30



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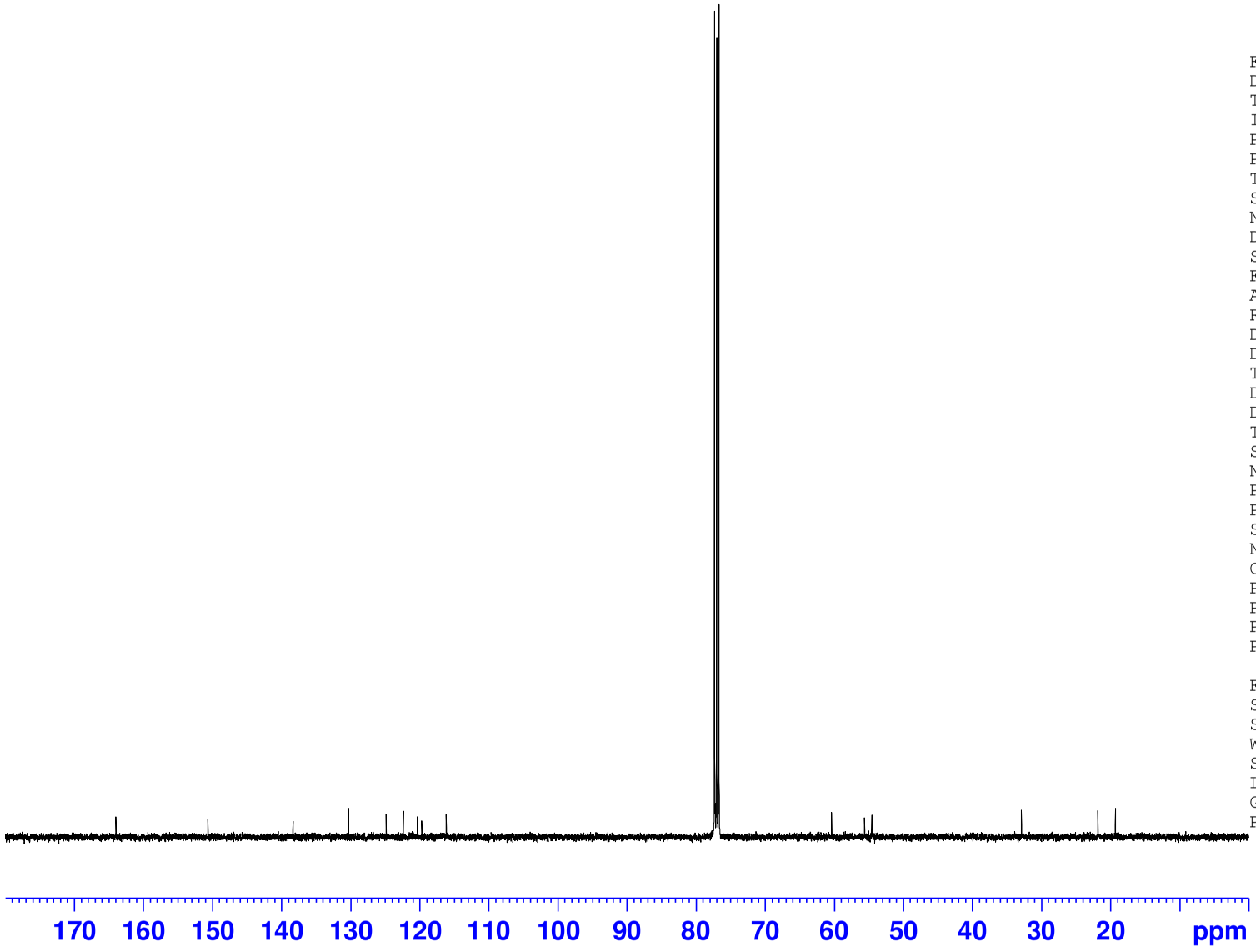


146

F2 - Acquisition Parameters

Date_	20180302	
Time	1.38	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

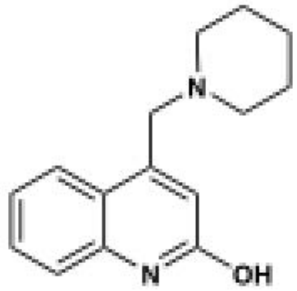
Faculty Group Callam

7.960
7.943
7.940
7.512
7.508
7.494
7.491
7.488
7.473
7.470
7.372
7.370
7.351
7.349
7.234
7.231
7.216
7.213
7.211
7.196
7.193
6.800

3.657
3.655

2.479

1.598
1.584
1.577
1.470
1.458



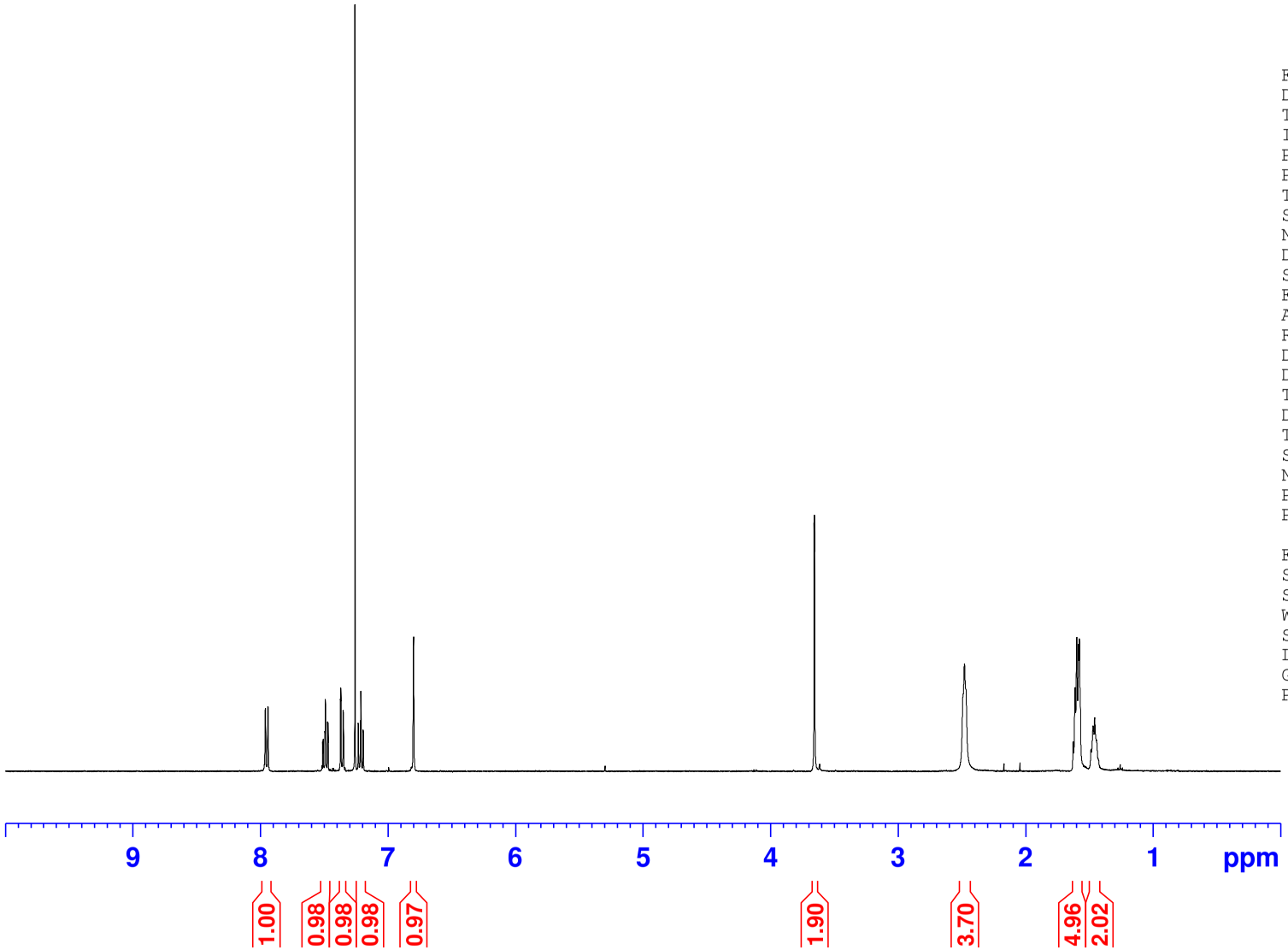
2.11

F2 - Acquisition Parameters

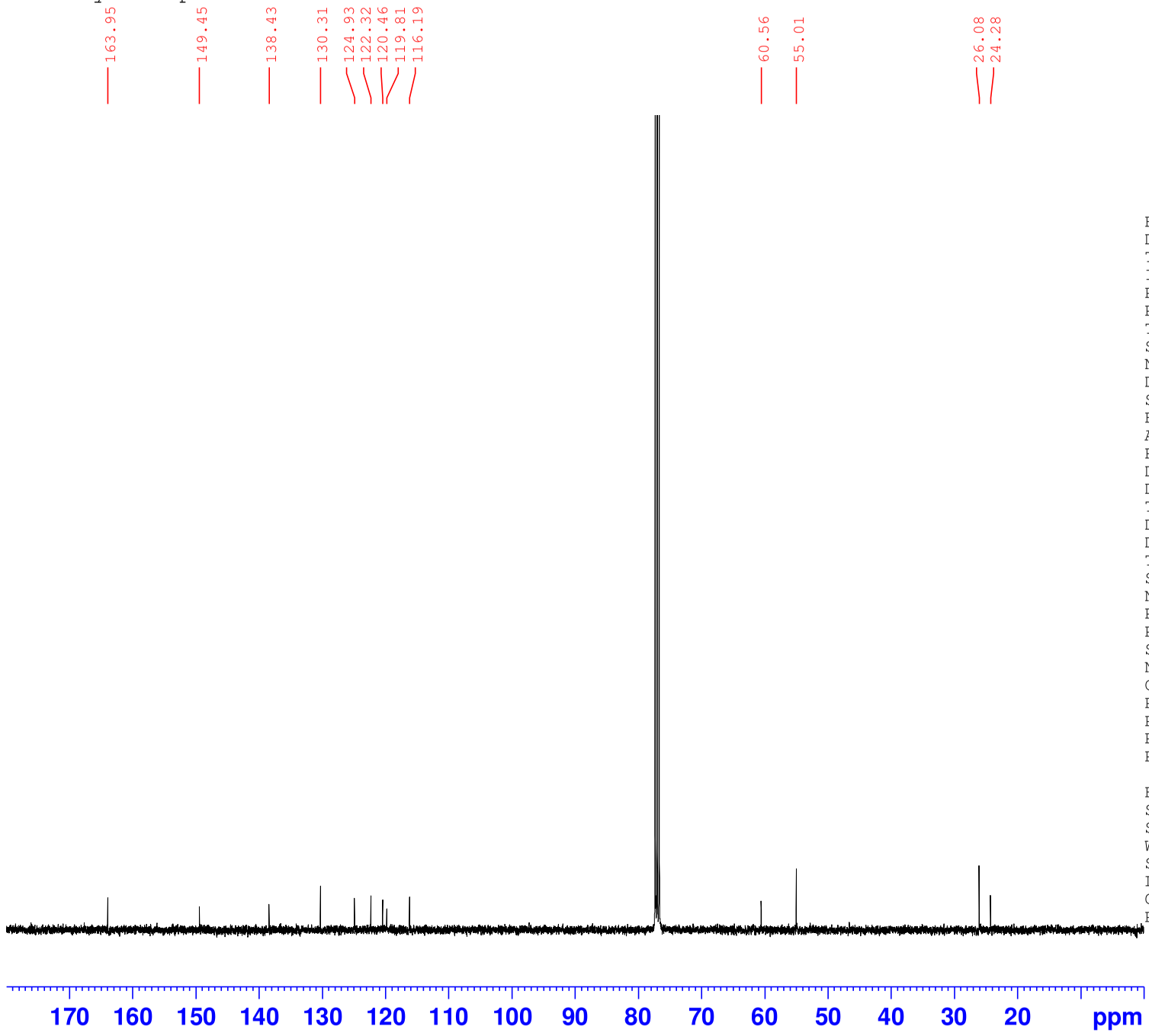
Date_	20180313	
Time	7.44	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

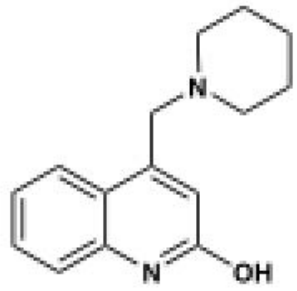
F2 - Processing parameters

SI	65536	
SF	400.1700104	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	



Faculty Group Callam





2.11

F2 - Acquisition Parameters

Date_	20180317	
Time	6.45	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

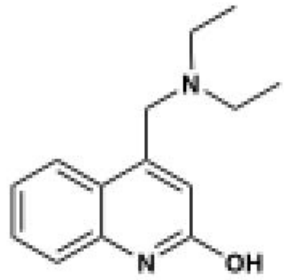
Faculty Group Callam

7.964 7.946 7.944 7.501 7.498 7.483 7.480 7.477 7.463 7.459 7.391 7.389 7.370 7.368 7.220 7.217 7.202 7.200 7.197 7.182 7.179 6.880

3.772 3.769

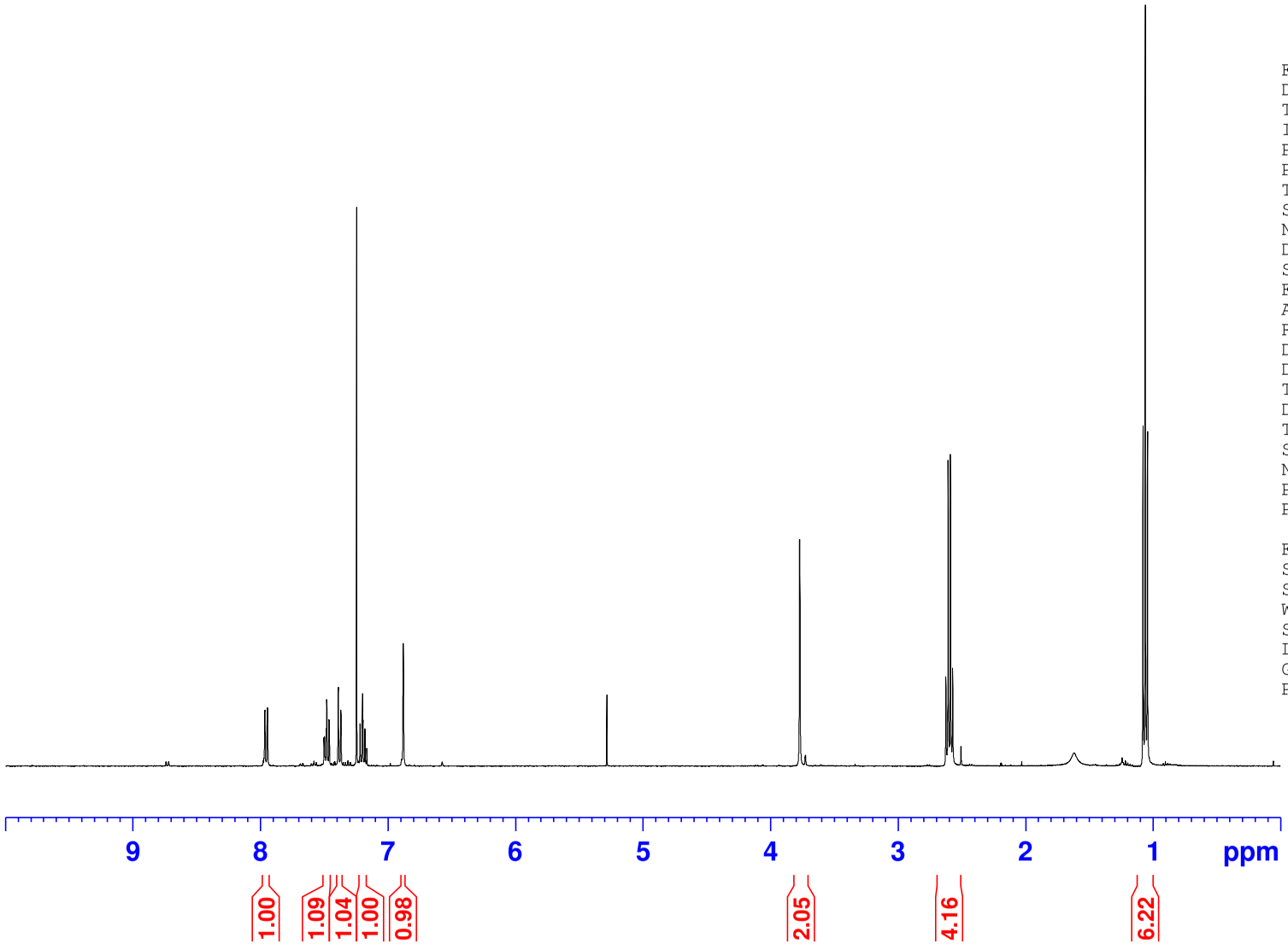
2.625 2.607 2.589 2.571

1.079 1.061 1.043



2.12

149



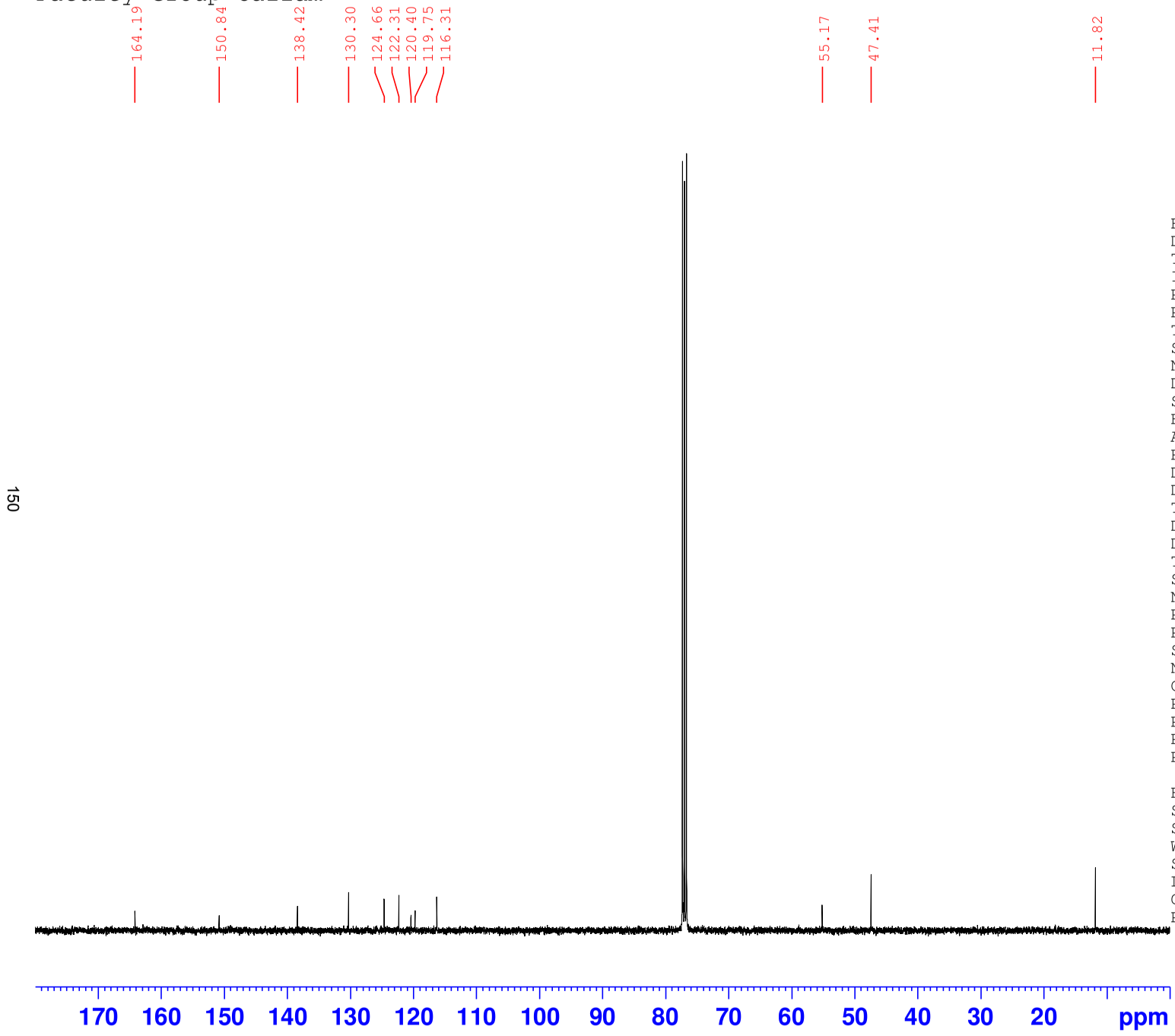
F2 - Acquisition Parameters

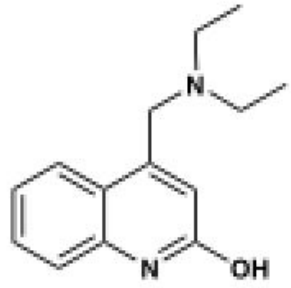
Date_	20180403	
Time	13.14	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700155	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





2.12

F2 - Acquisition Parameters

Date_	20180404	
Time	7.52	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

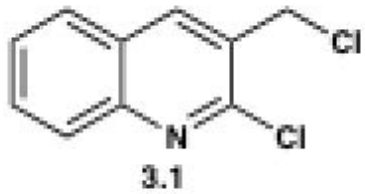
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

APPENDIX B

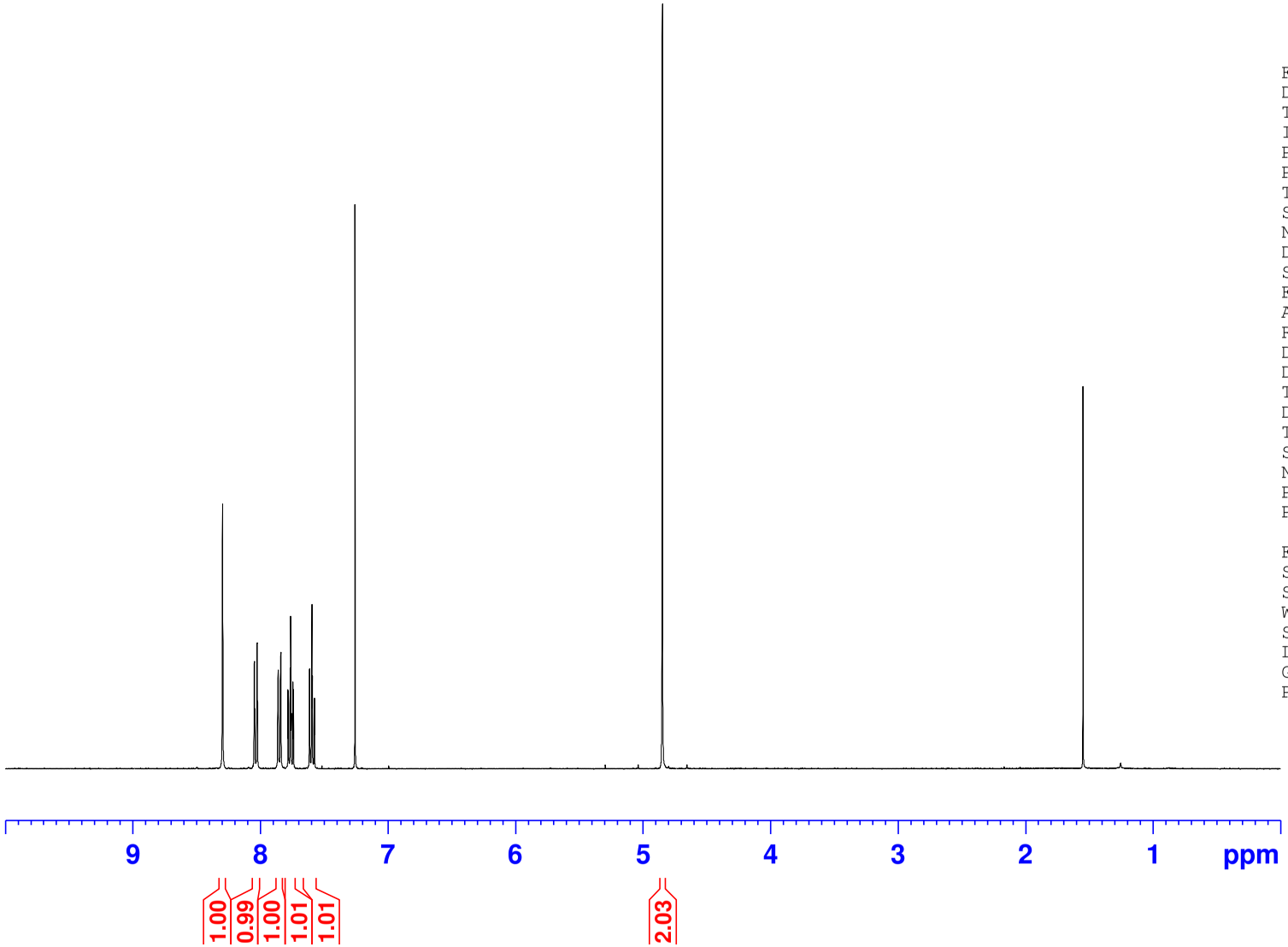
SELECT ^{1}HNMR AND ^{13}CNMR DATA FROM CHAPTER 3

Faculty Group Callam

8.046
8.028
8.025
7.864
7.860
7.843
7.840
7.784
7.780
7.766
7.763
7.759
7.745
7.741
7.617
7.614
7.599
7.596
7.593
7.579
7.576
4.848



3.1



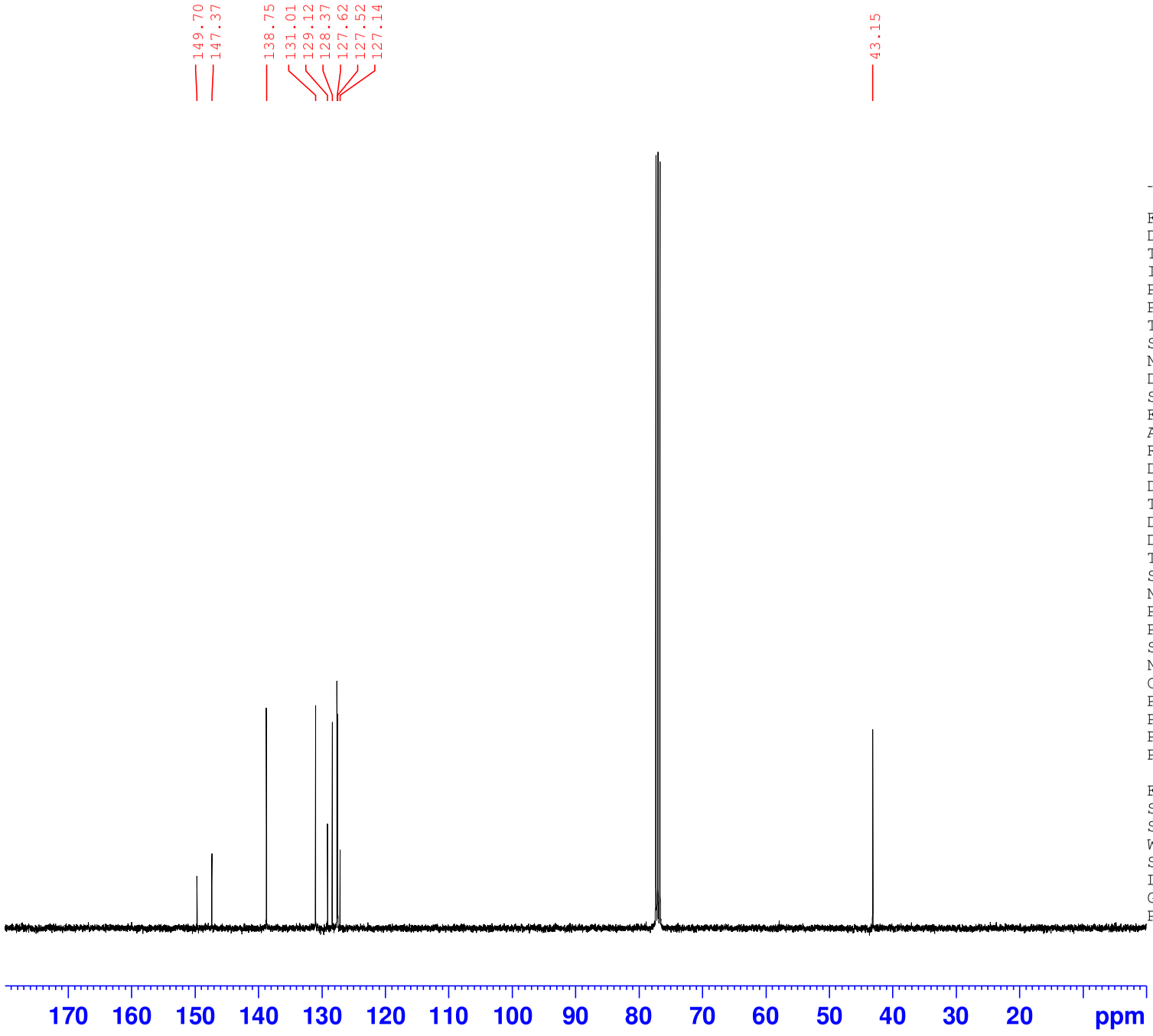
F2 - Acquisition Parameters

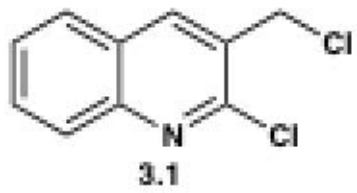
Date_	20180313	
Time	7.39	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	305.62	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700107	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





3.1

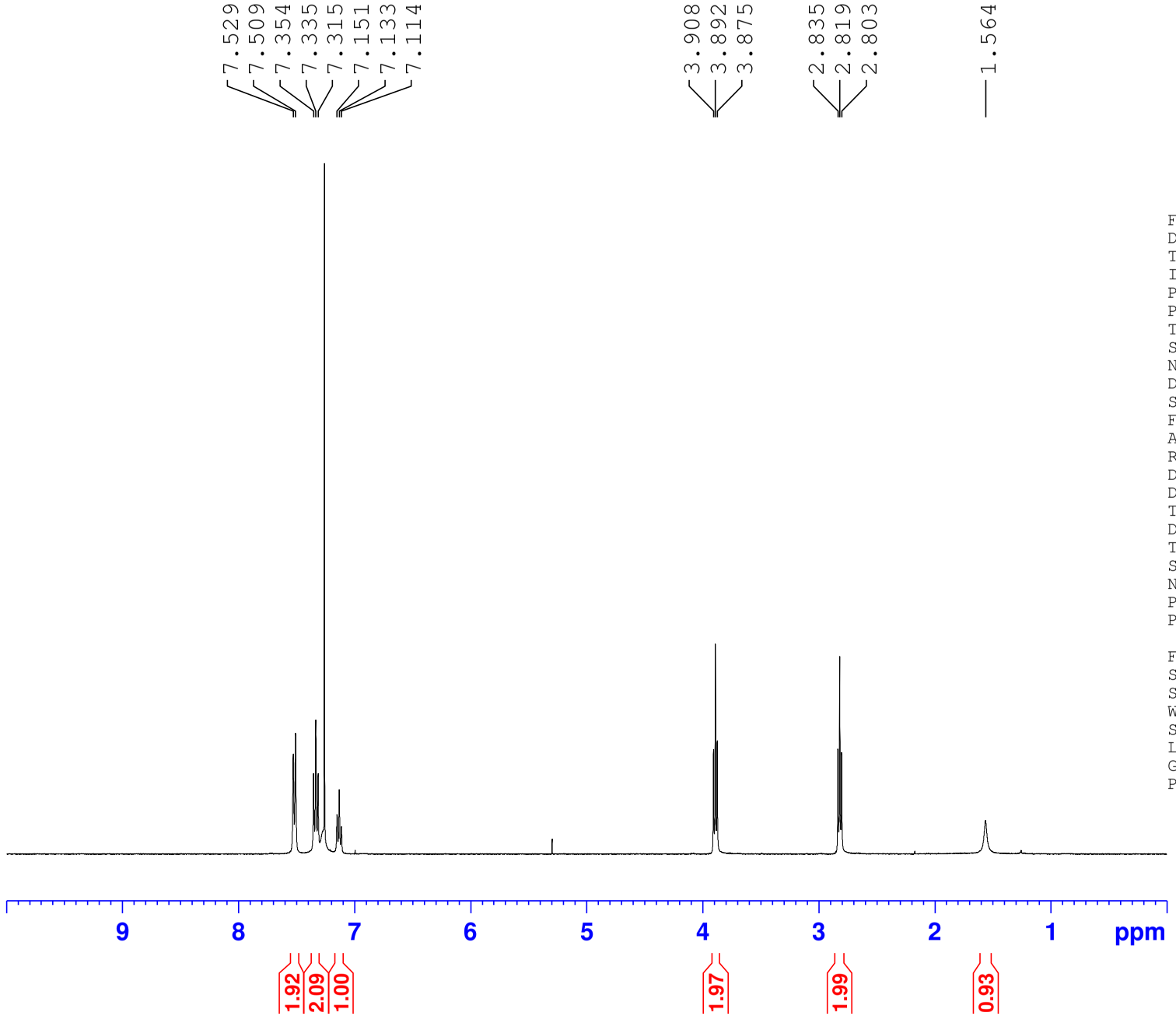
F2 - Acquisition Parameters

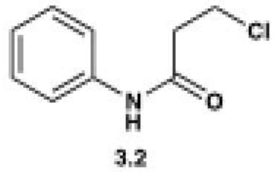
Date_	20180404	
Time	6.49	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam





3.2

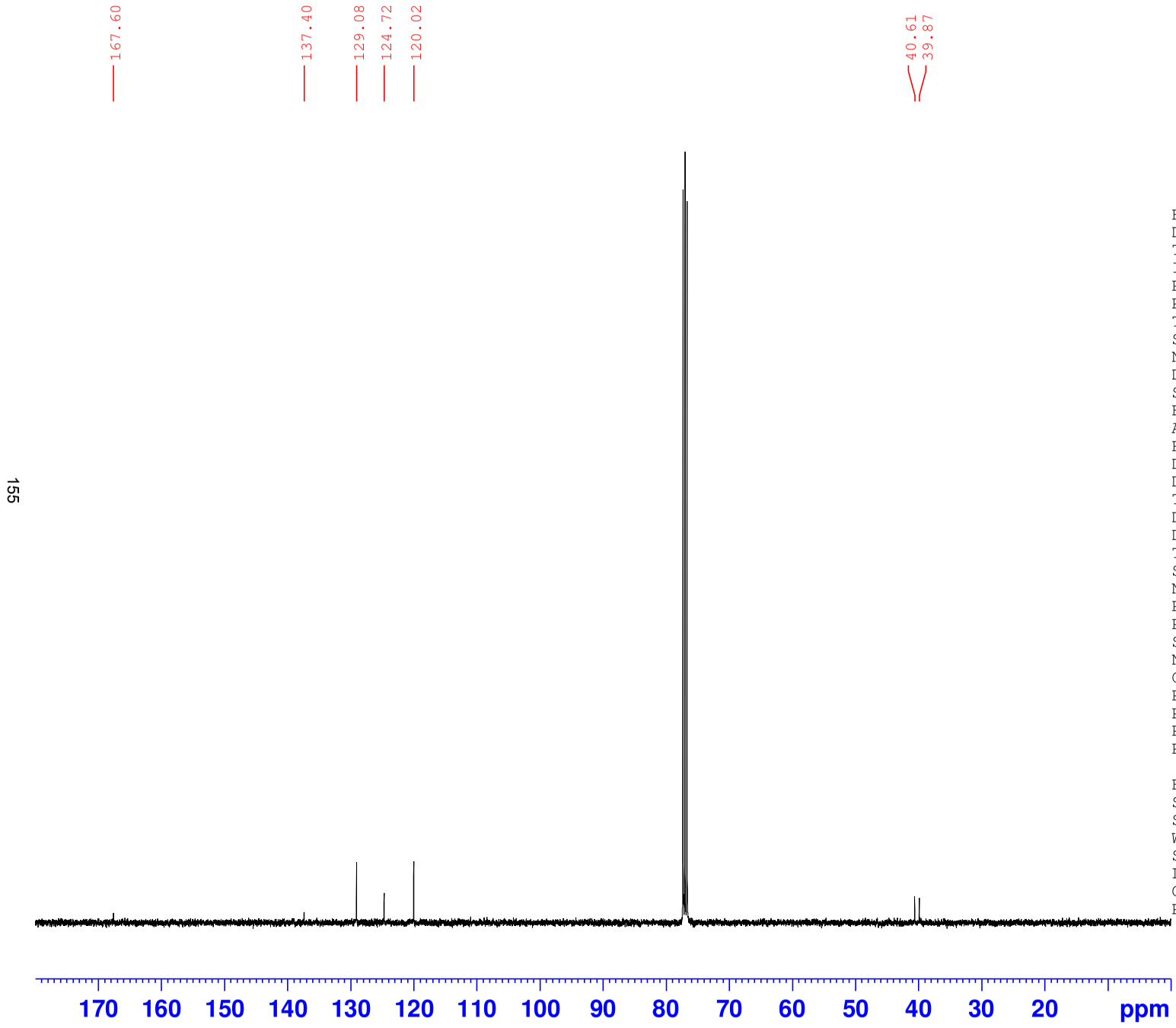
F2 - Acquisition Parameters

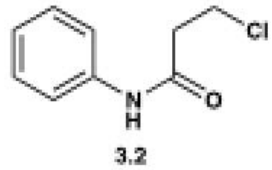
Date_	20180228	
Time	18.11	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700101	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam



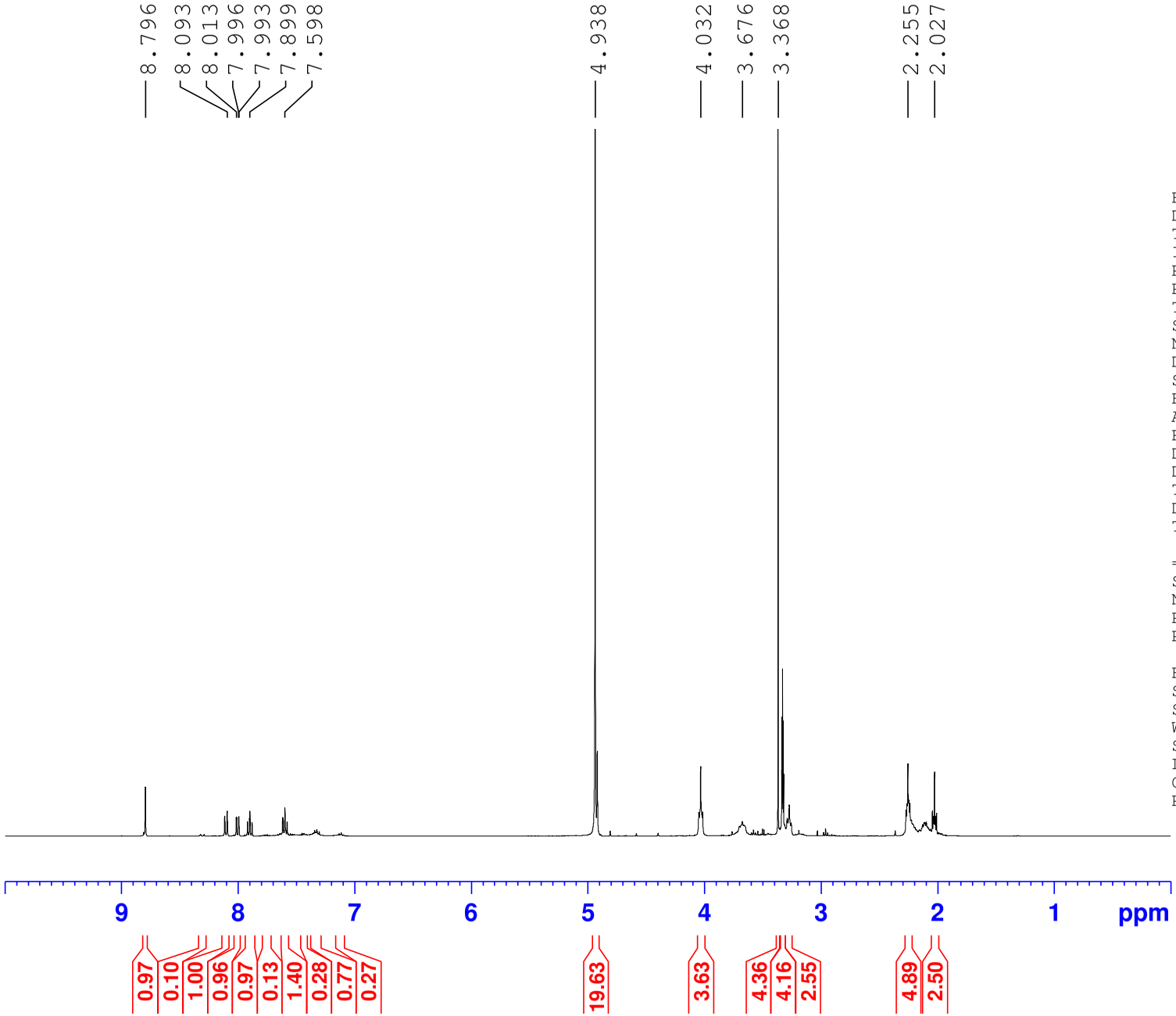


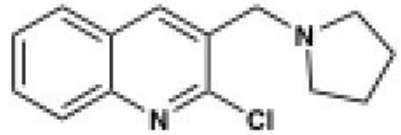
3.2

```
F2 - Acquisition Parameters
Date_           20180302
Time                3.44 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ             1.3631488 sec
RG                  2050
DW                20.800 usec
DE                 6.50 usec
TE                 300.5 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                 32768
SF           100.6228265 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Faculty Group Callam
PROTON_OSU MeOD {C:\Bruker\TopSpin3.0} sillart.1 90





3.3

F2 - Acquisition Parameters

Date_	20160717
Time	3.25
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zg30
TD	65536
SOLVENT	MeOD
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9845889 sec
RG	134.1
DW	60.800 usec
DE	6.50 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1

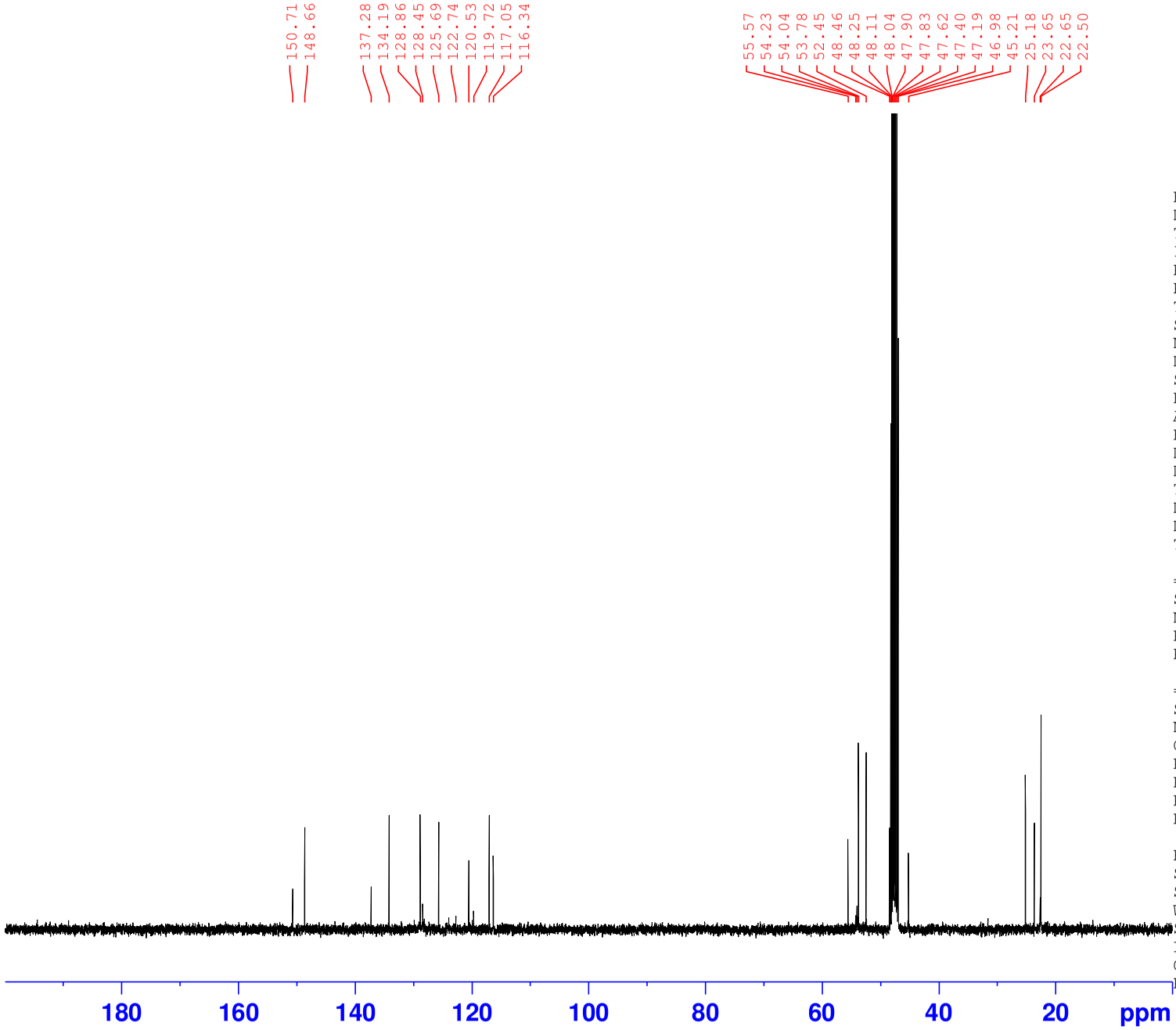
======== CHANNEL f1 ========

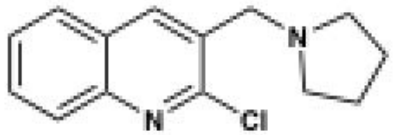
SFO1	400.1724712 MHz
NUC1	1H
P1	15.00 usec
PLW1	13.19999981 W

F2 - Processing parameters

SI	65536
SF	400.1700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Faculty Group Callam
C13CPD MeOD {C:\Bruker\TopSpin3.0} sillart.1 90





3.3

F2 - Acquisition Parameters

Date_	20160717
Time	4.26
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zgpg30
TD	65536
SOLVENT	MeOD
NS	1024
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631488 sec
RG	2050
DW	20.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1

======== CHANNEL f1 ========

SFO1	100.6328883 MHz
NUC1	13C
P1	9.50 usec
PLW1	53.29999924 W

======== CHANNEL f2 ========

SFO2	400.1716007 MHz
NUC2	1H
CPDPRG[2	waltz16
PCPD2	80.00 usec
PLW2	14.60000038 W
PLW12	0.51327997 W
PLW13	0.32850000 W

F2 - Processing parameters

SI	32768
SF	100.6228265 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

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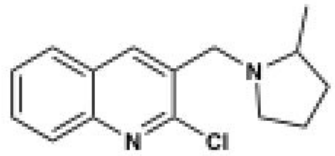
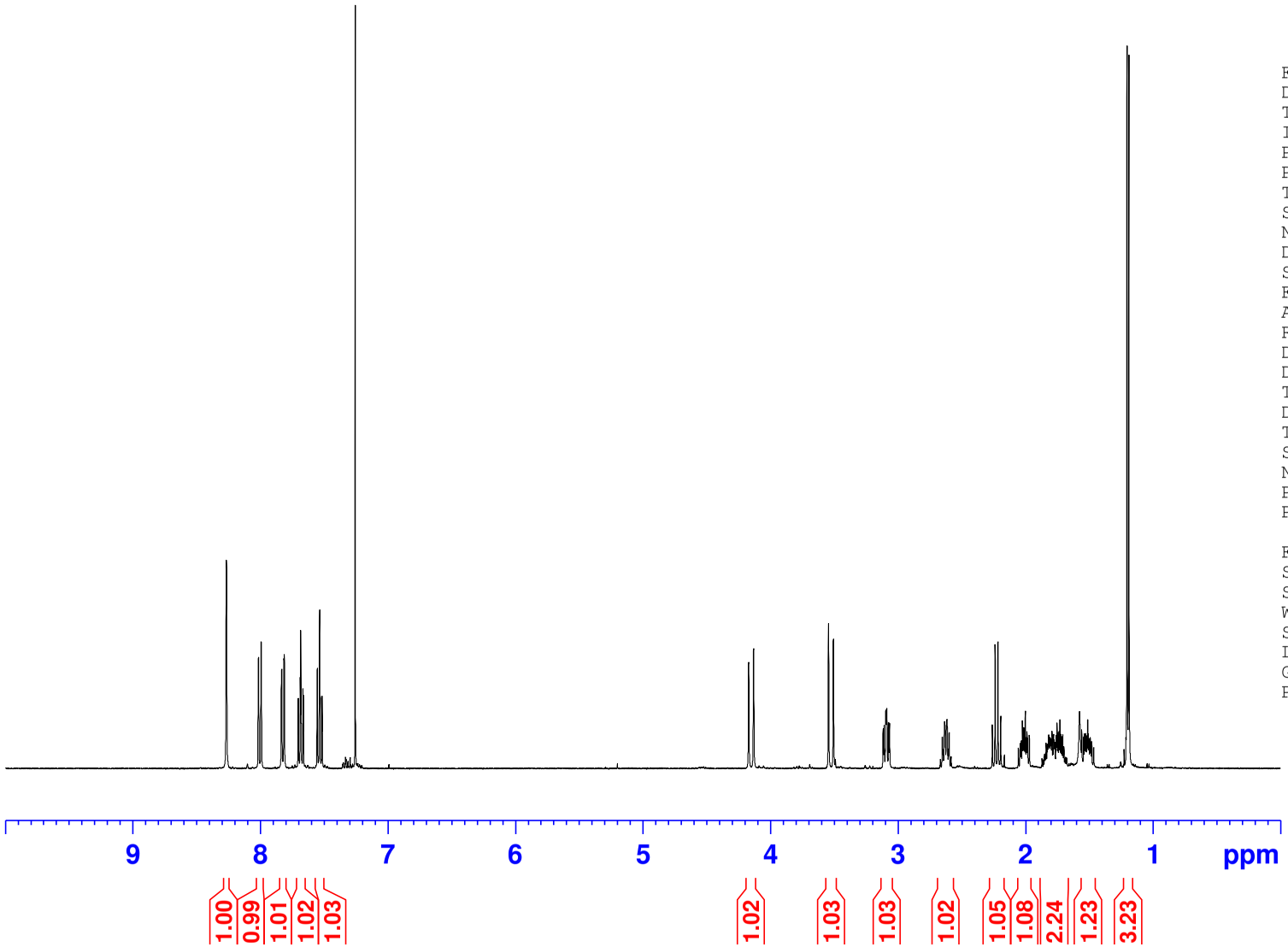
8.267 8.015 7.996 7.994 7.836 7.833 7.816 7.813 7.705 7.702 7.688 7.684 7.681 7.667 7.663 7.557 7.554 7.539 7.537 7.534 7.519 7.516 4.172 4.170 4.134 4.132 3.544 3.506 3.109 3.094 3.087 3.074 3.066 2.635 2.616 2.261 2.239 2.216 2.195 2.025 2.001 1.512 1.205 1.190



3.4



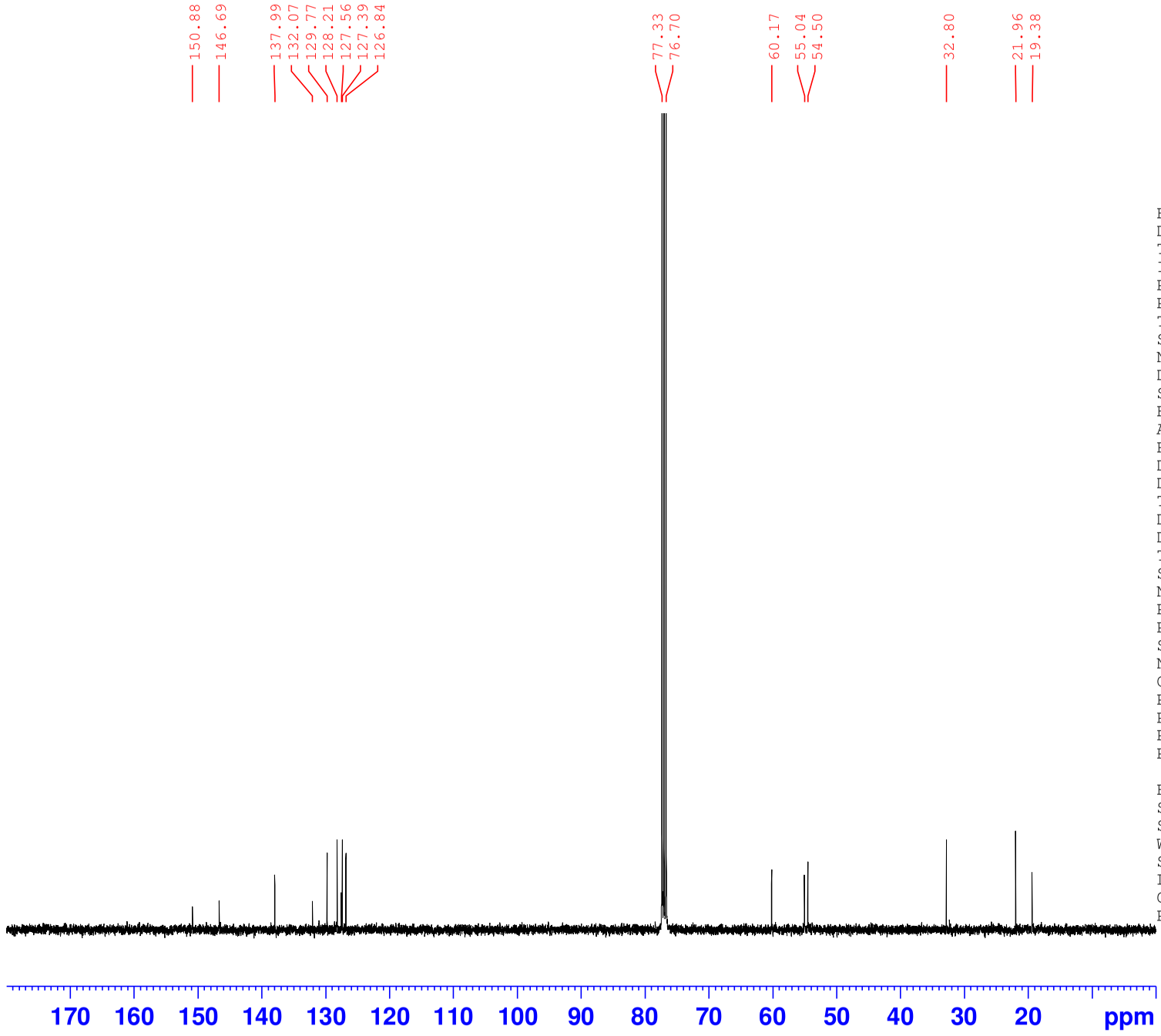
F2 - Acquisition Parameters

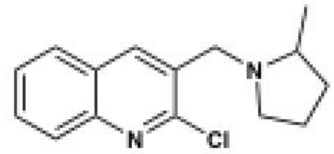
Date_	20180313	
Time	8.01	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700112	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





3.4

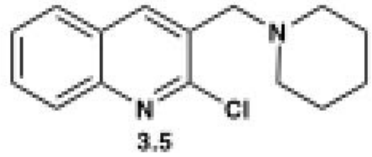
```
F2 - Acquisition Parameters
Date_           20180317
Time                9.56 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1                13C
P1                9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

Faculty Group Callam

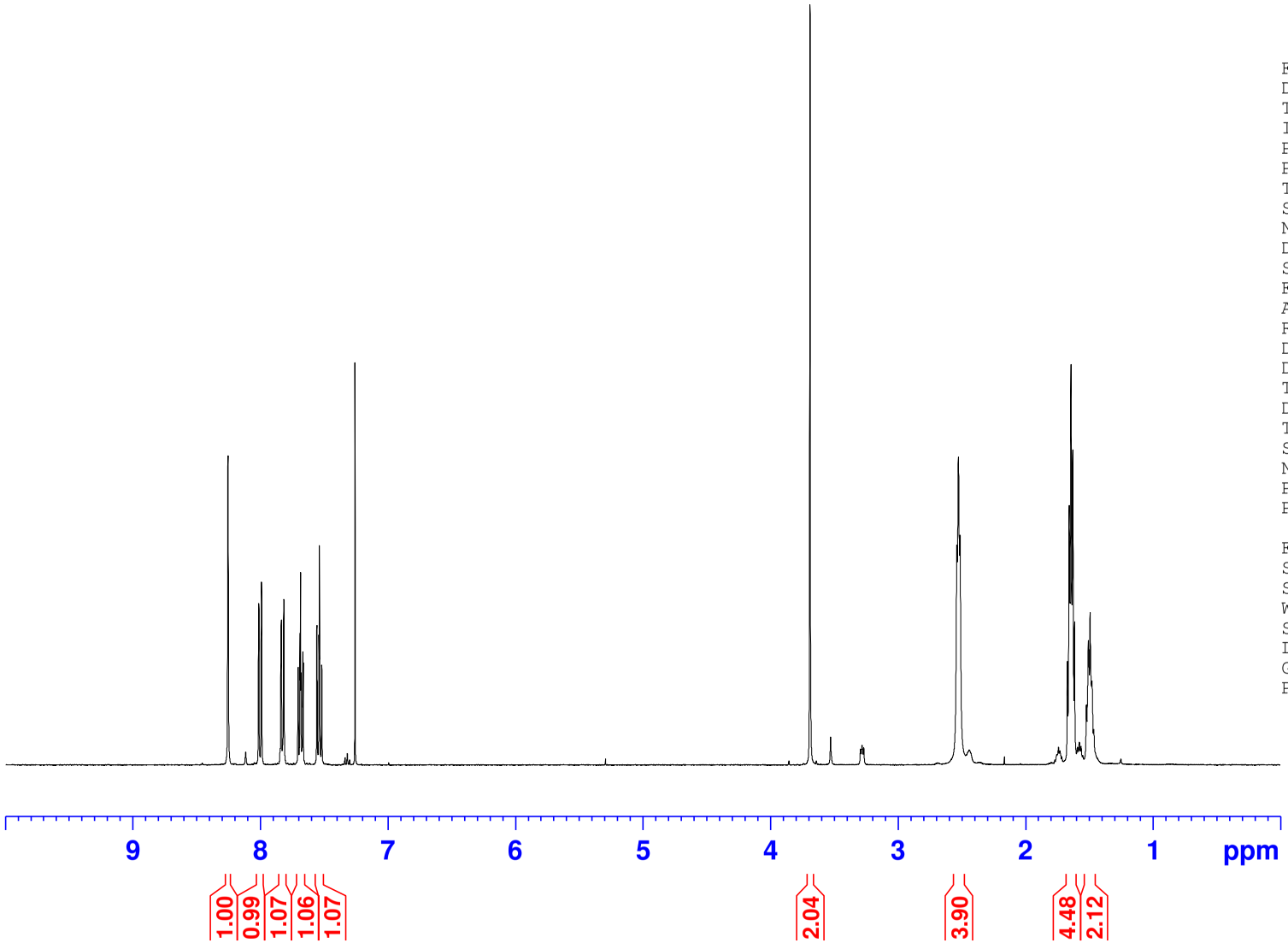
8.013
7.993
7.992
7.839
7.836
7.818
7.816
7.706
7.703
7.689
7.685
7.682
7.668
7.664
7.558
7.555
7.541
7.538
7.535
7.521
7.518

3.691
3.689

2.539
2.526
2.514
1.672
1.657
1.643
1.629
1.616
1.507
1.494
1.479



3.5



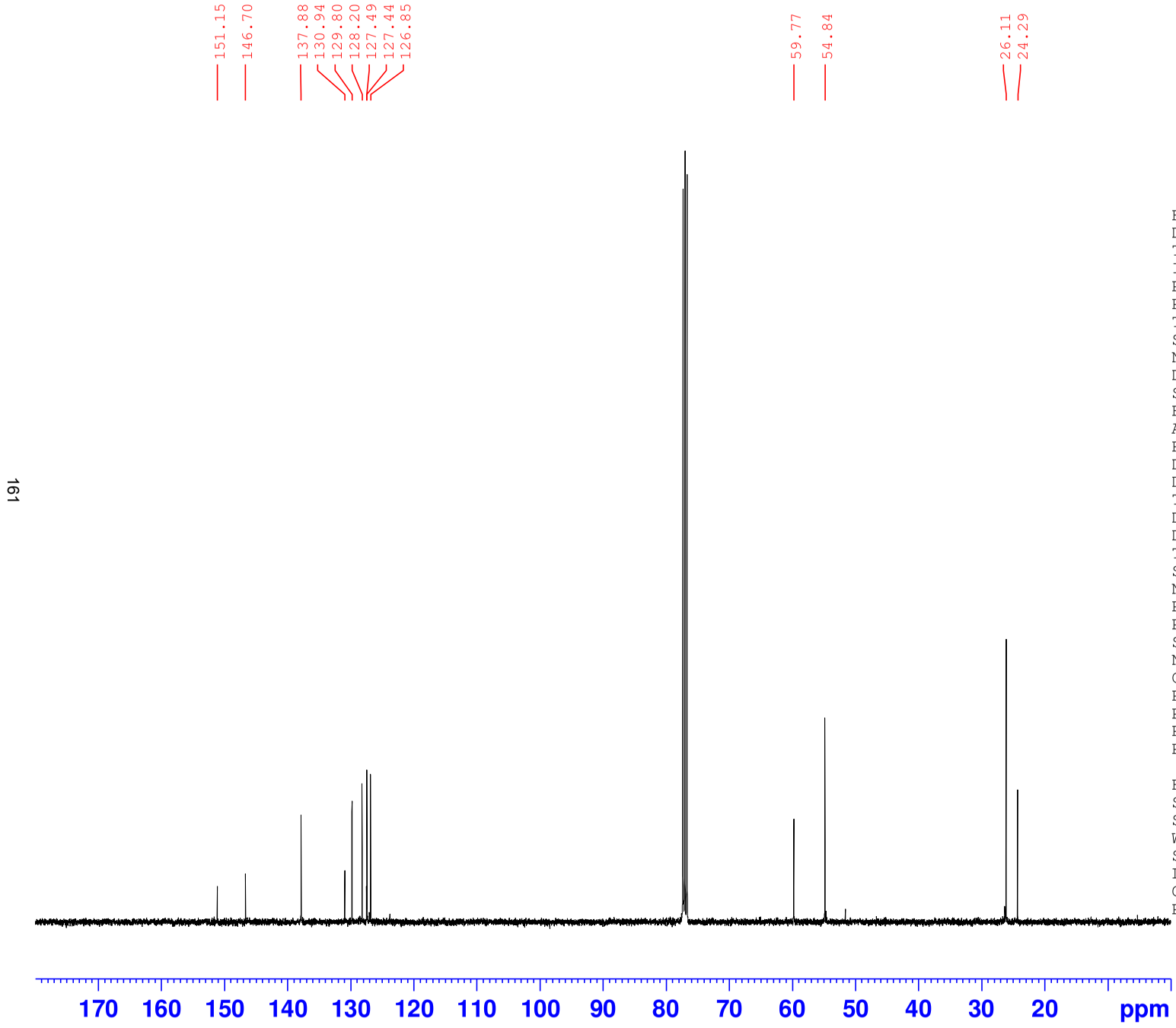
F2 - Acquisition Parameters

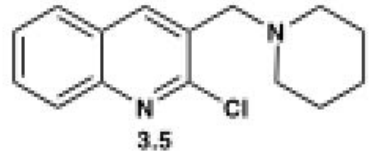
Date_	20180315	
Time	4.06	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700107	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





3.5

F2 - Acquisition Parameters

Date_	20180315	
Time	4.03	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

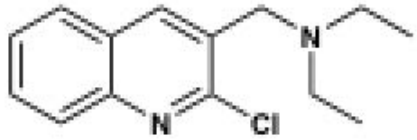
Faculty Group Callam

8.017
7.997
7.995
7.841
7.838
7.821
7.818
7.704
7.701
7.687
7.683
7.680
7.666
7.662
7.559
7.556
7.542
7.539
7.536
7.521
7.518

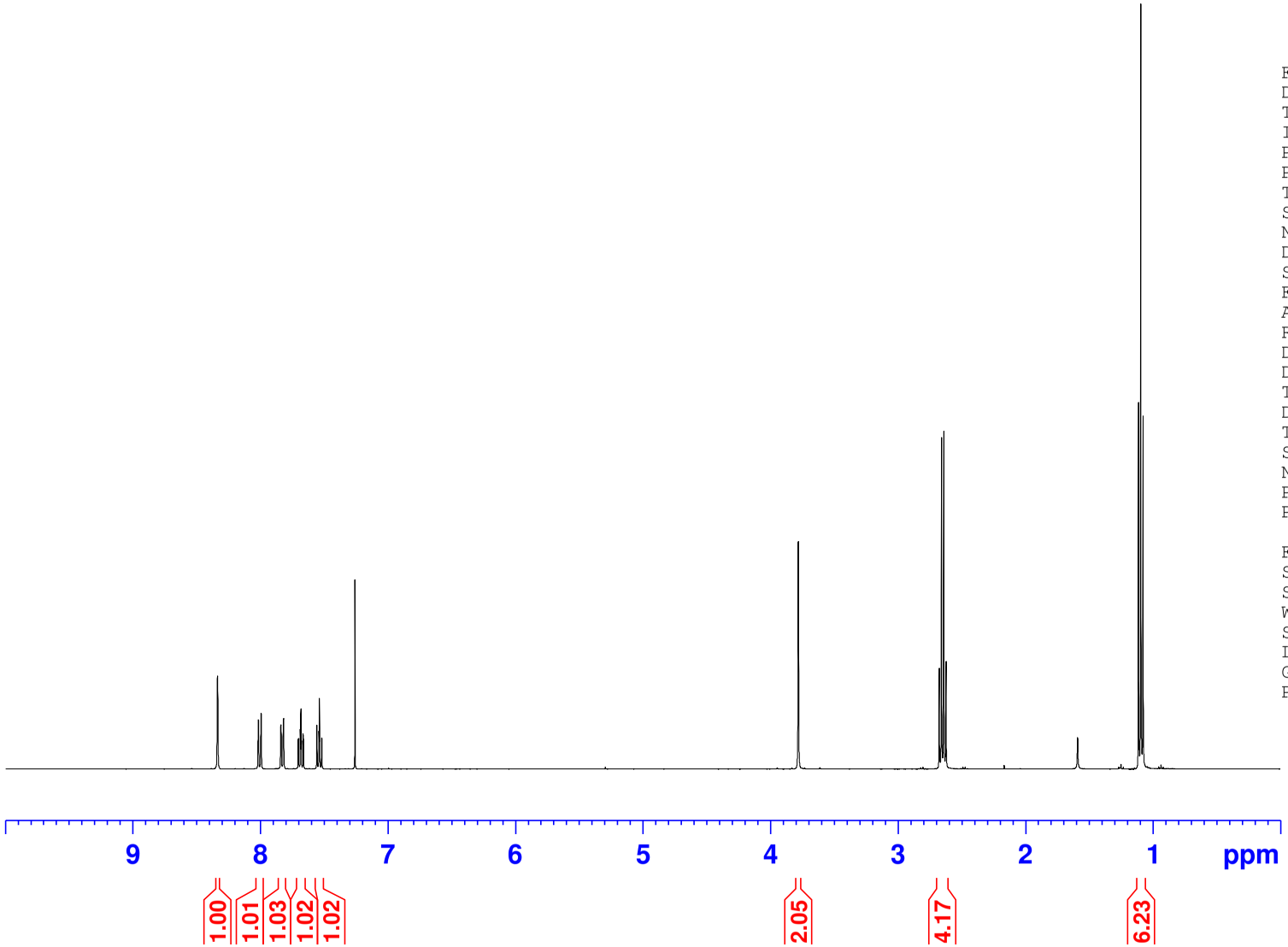
3.783
3.781

2.677
2.659
2.641
2.624

1.114
1.096
1.078



3.6



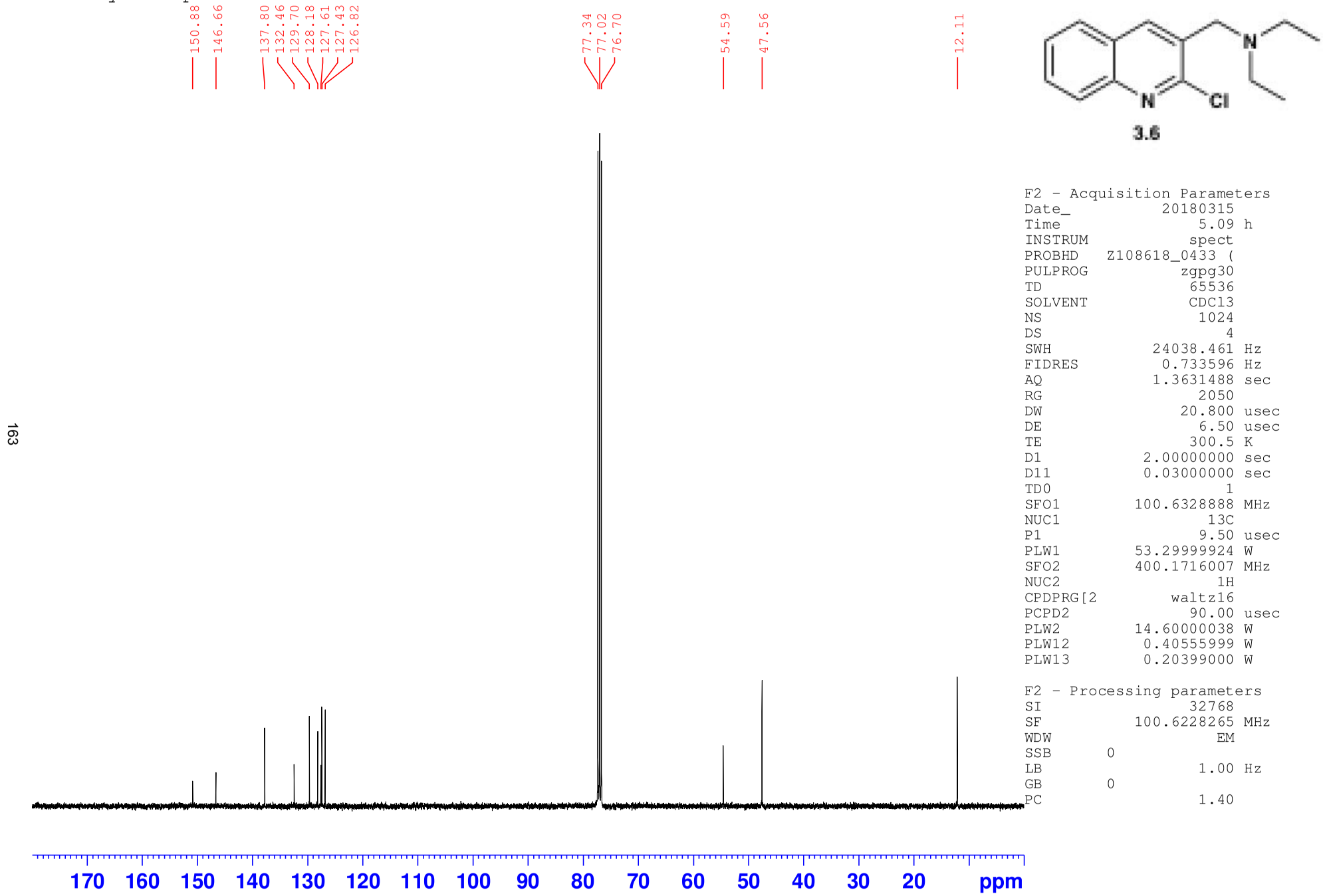
F2 - Acquisition Parameters

Date_	20180314	
Time	18.53	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700100	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam



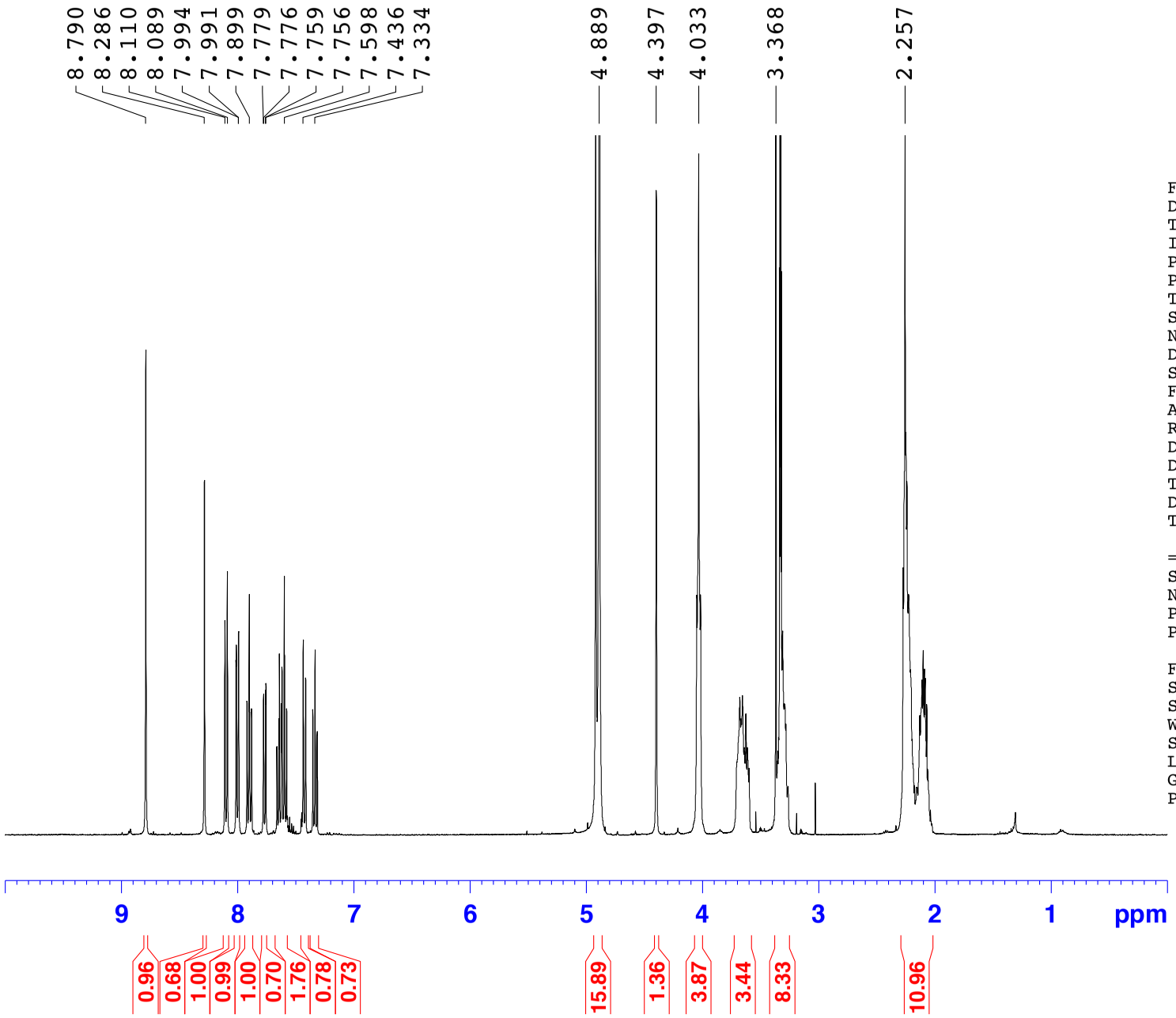
F2 - Acquisition Parameters

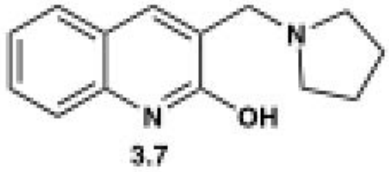
Date_	20180315	
Time	5.09	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam
PROTON_OSU MeOD {C:\Bruker\TopSpin3.0} sillart.1 83





3.7

F2 - Acquisition Parameters

Date_	20160705
Time	20.35
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zg30
TD	65536
SOLVENT	MeOD
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	120.36
DW	60.800 usec
DE	6.50 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1

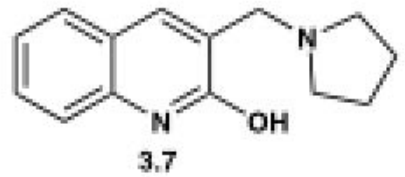
======== CHANNEL f1 ========

SFO1	400.1724712 MHz
NUC1	1H
P1	15.00 usec
PLW1	13.19999981 W

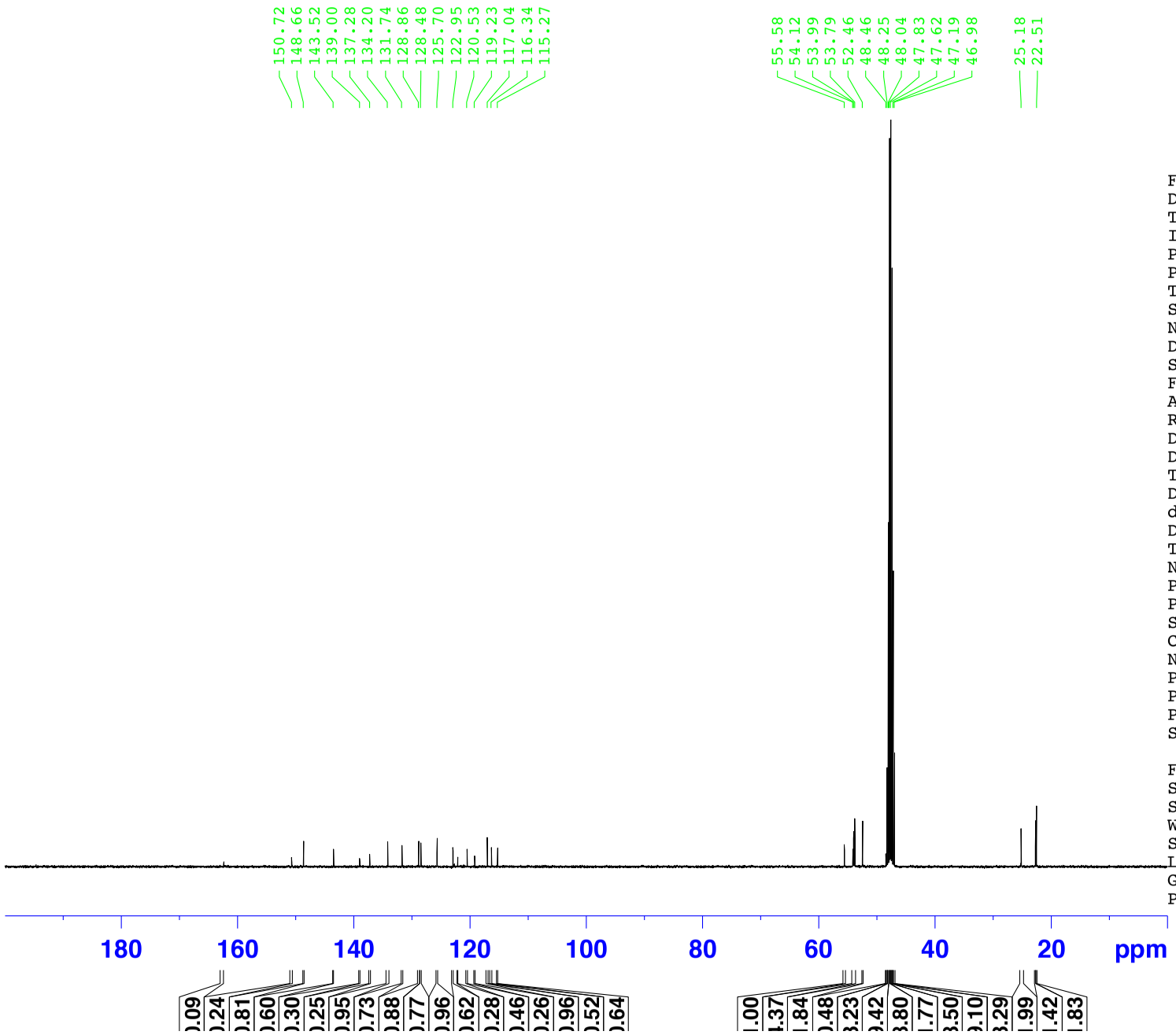
F2 - Processing parameters

SI	65536
SF	400.1700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Faculty Group Callam
C13CPD MeOD {C:\Bruker\TopSpin3.0} sillart.1 83



3.7



165

F2 - Acquisition Parameters

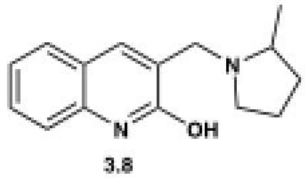
Date_	20160706
Time	4.00
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zgpg30
TD	65536
SOLVENT	MeOD
NS	1024
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	2050
DW	20.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
d11	0.03000000 sec
DELTA	1.89999998 sec
TD0	1
NUC1	13C
P1	9.50 usec
PLW1	53.29999924 W
SFO1	100.6328883 MHz
CPDPRG2	
NUC2	1H
PLW2	14.60000038 W
PLW12	0.51327997 W
PLW13	0.32850000 W
SFO2	400.1716007 MHz

F2 - Processing parameters

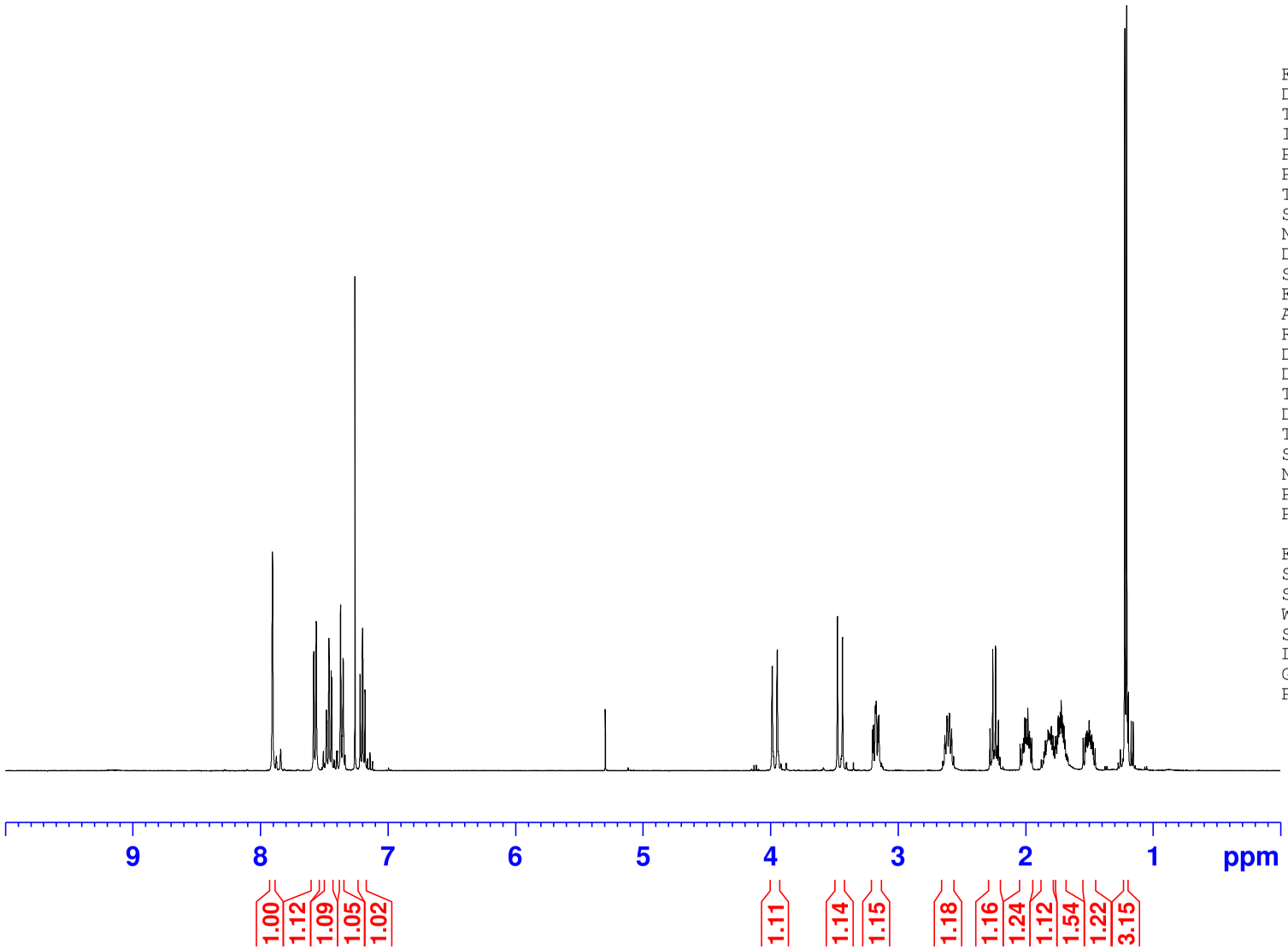
SI	32768
SF	100.6228265 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

Faculty Group Callam

7.906 7.584 7.582 7.562 7.483 7.480 7.465 7.462 7.459 7.445 7.441 7.372 7.351 7.219 7.216 7.201 7.199 7.182 7.179 3.989 3.985 3.949 3.946 3.476 3.474 3.437 3.434 3.177 3.172 3.156 3.149 2.616 2.612 2.600 2.597 2.257 2.235 2.213 2.006 1.994 1.982 1.720 1.222 1.207



3.8



166

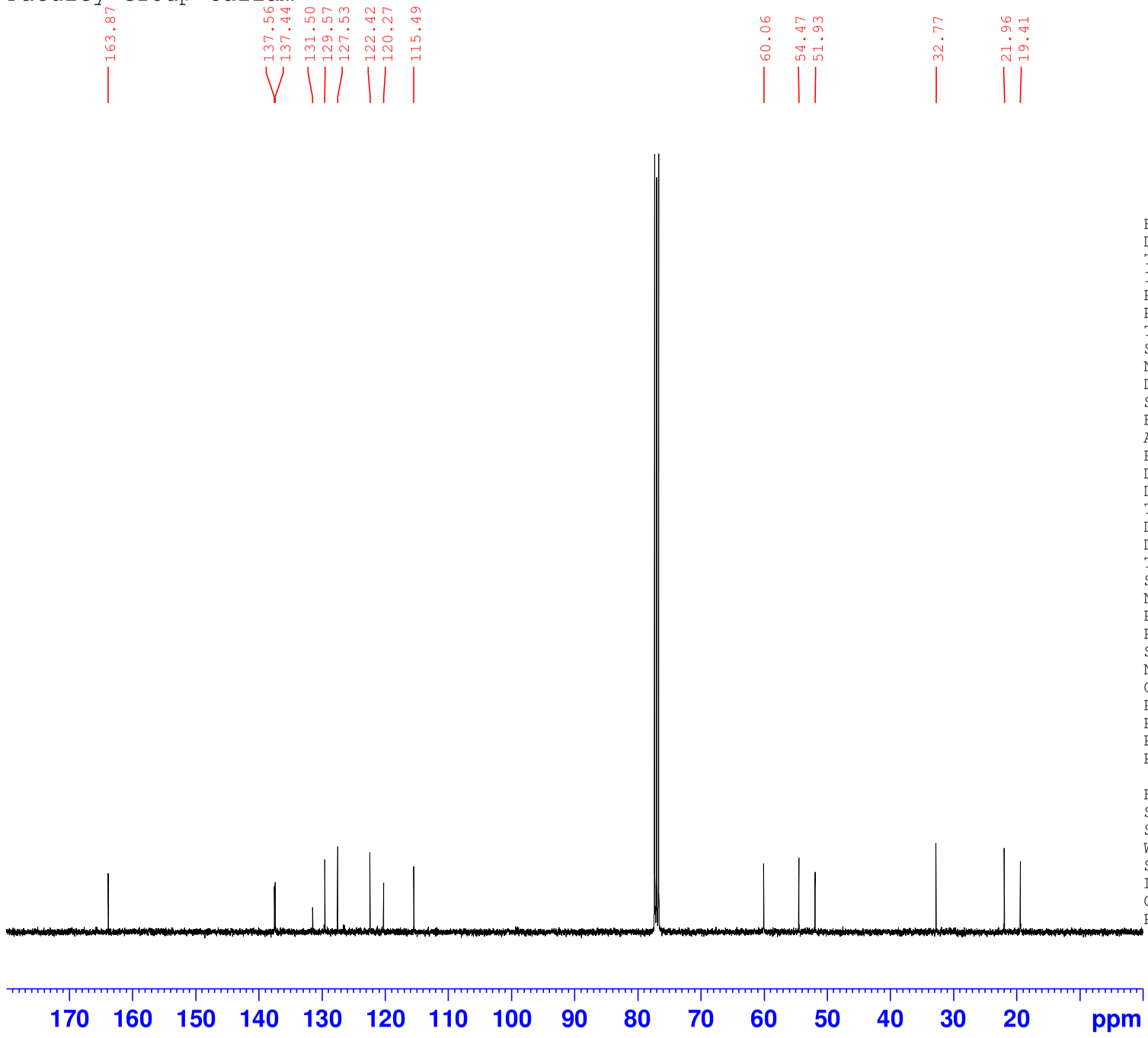
F2 - Acquisition Parameters

Date_	20180403	
Time	12.51	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	188.13	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700102	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

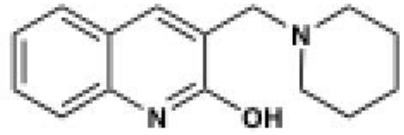
Faculty Group Callam



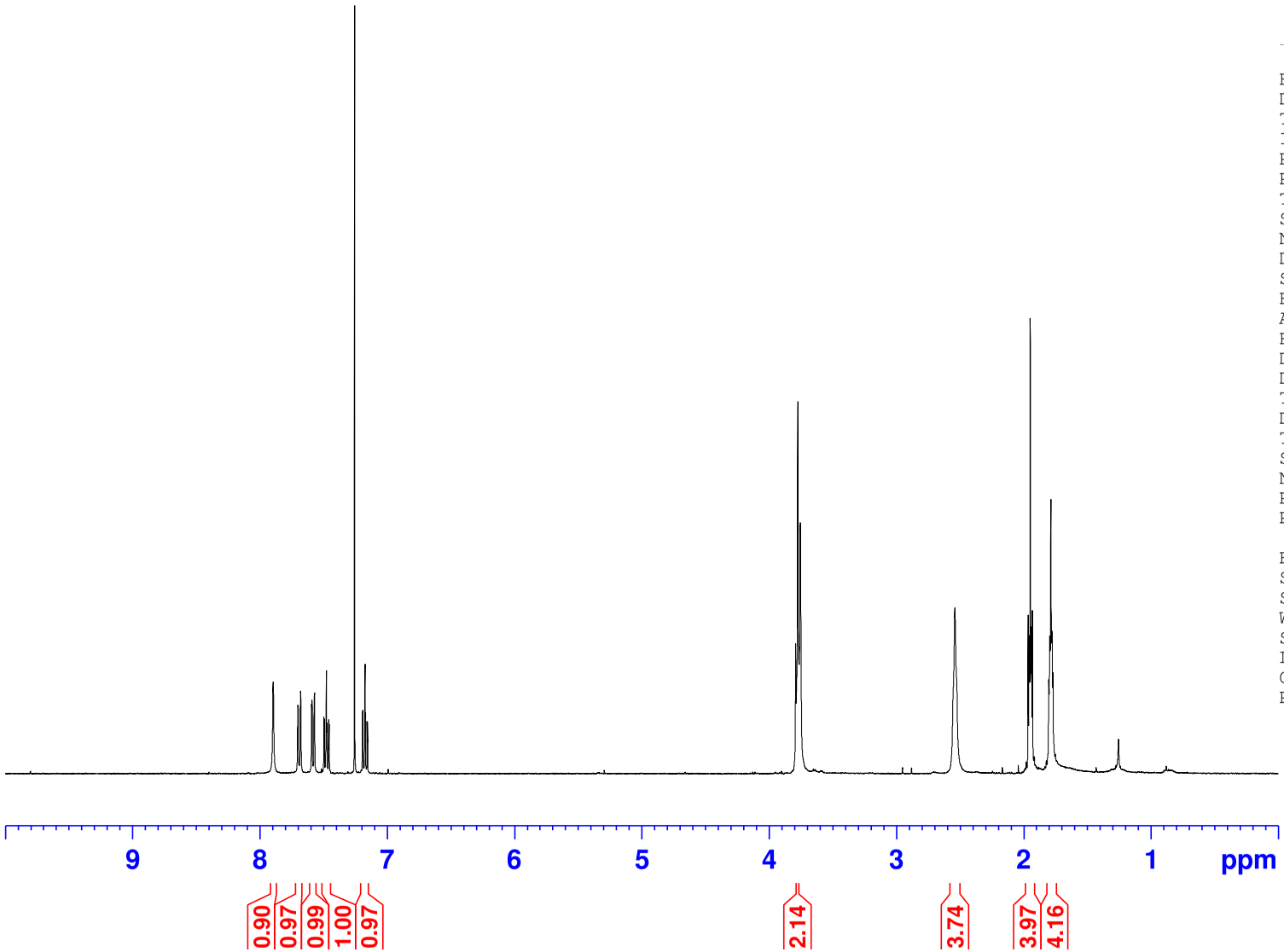

```
F2 - Acquisition Parameters
Date_           20180404
Time                3.39 h
INSTRUM             spect
PROBHD   Z108618_0433 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ             1.3631488 sec
RG                  2050
DW                20.800 usec
DE                 6.50 usec
TE                300.5 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                 32768
SF           100.6228265 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Faculty Group Callam

7.923 7.729 7.708 7.621 7.618 7.601 7.598 7.526 7.522 7.508 7.505 7.501 7.487 7.483 7.284 7.222 7.219 7.204 7.202 7.199 7.185 7.182 3.818 3.807 3.802 3.796 3.791 3.784 3.781 2.567 1.992 1.983 1.975 1.968 1.958 1.944 1.849 1.830 1.822 1.813 1.805 1.797 1.778 1.281



3.9



168

F2 - Acquisition Parameters

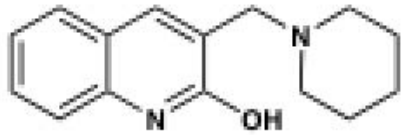
Date_	20180314	
Time	19.09	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

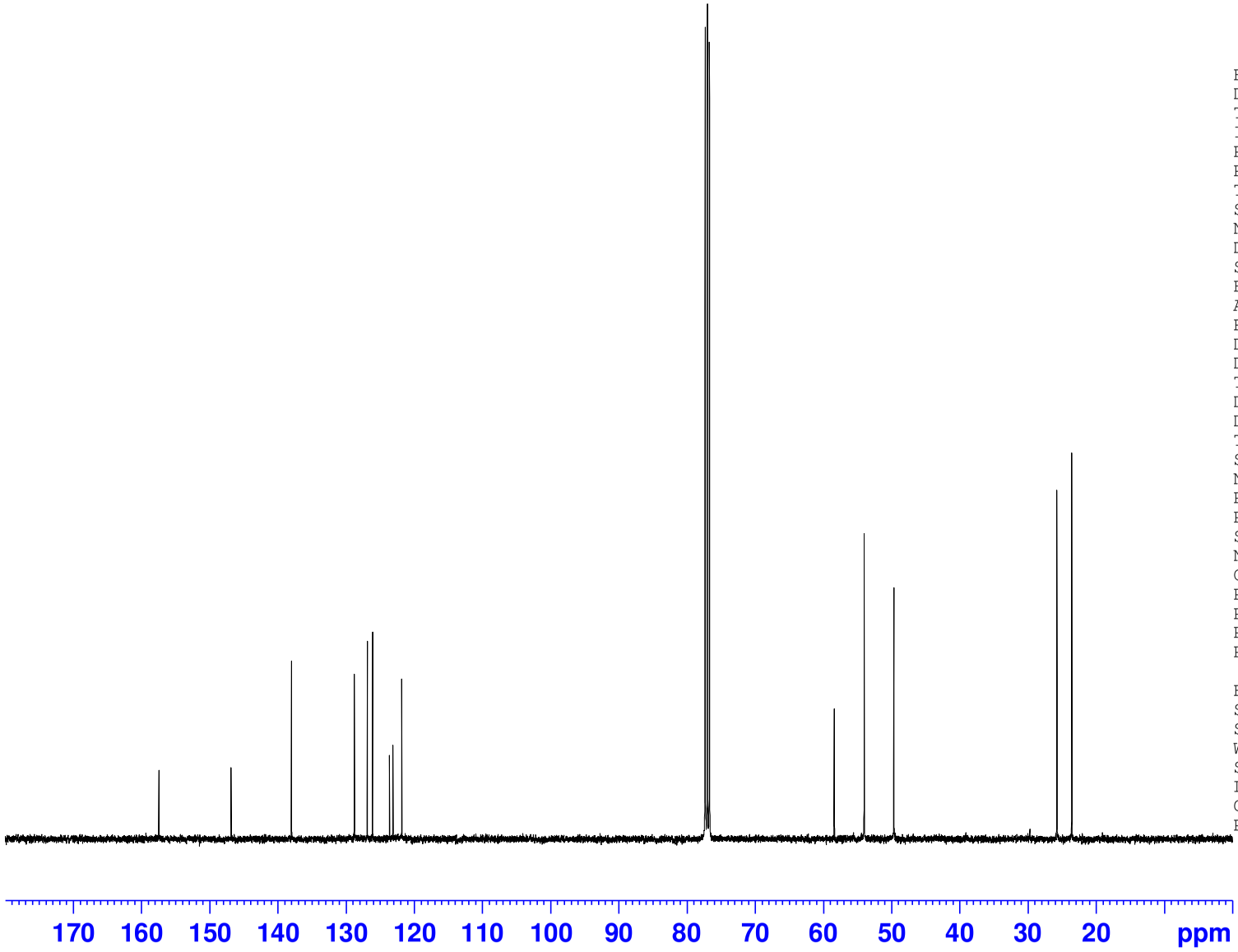
SI	65536	
SF	400.1700107	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

157.47
146.88
138.02
128.79
126.90
126.12
123.64
123.14
121.85
58.41
54.02
49.66
29.72
25.74
23.58



3.9



169

```
F2 - Acquisition Parameters
Date_           20180404
Time                5.45 h
INSTRUM            spect
PROBHD   Z108618_0433 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1               13C
P1                9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2             90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

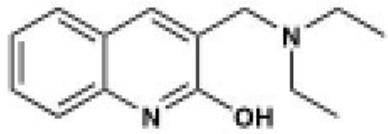
Faculty Group Callam

7.961 7.592 7.589 7.572 7.569 7.483 7.480 7.465 7.462 7.459 7.445 7.441 7.355 7.334 7.225 7.222 7.206 7.205 7.202 7.187 7.184

3.656 3.652

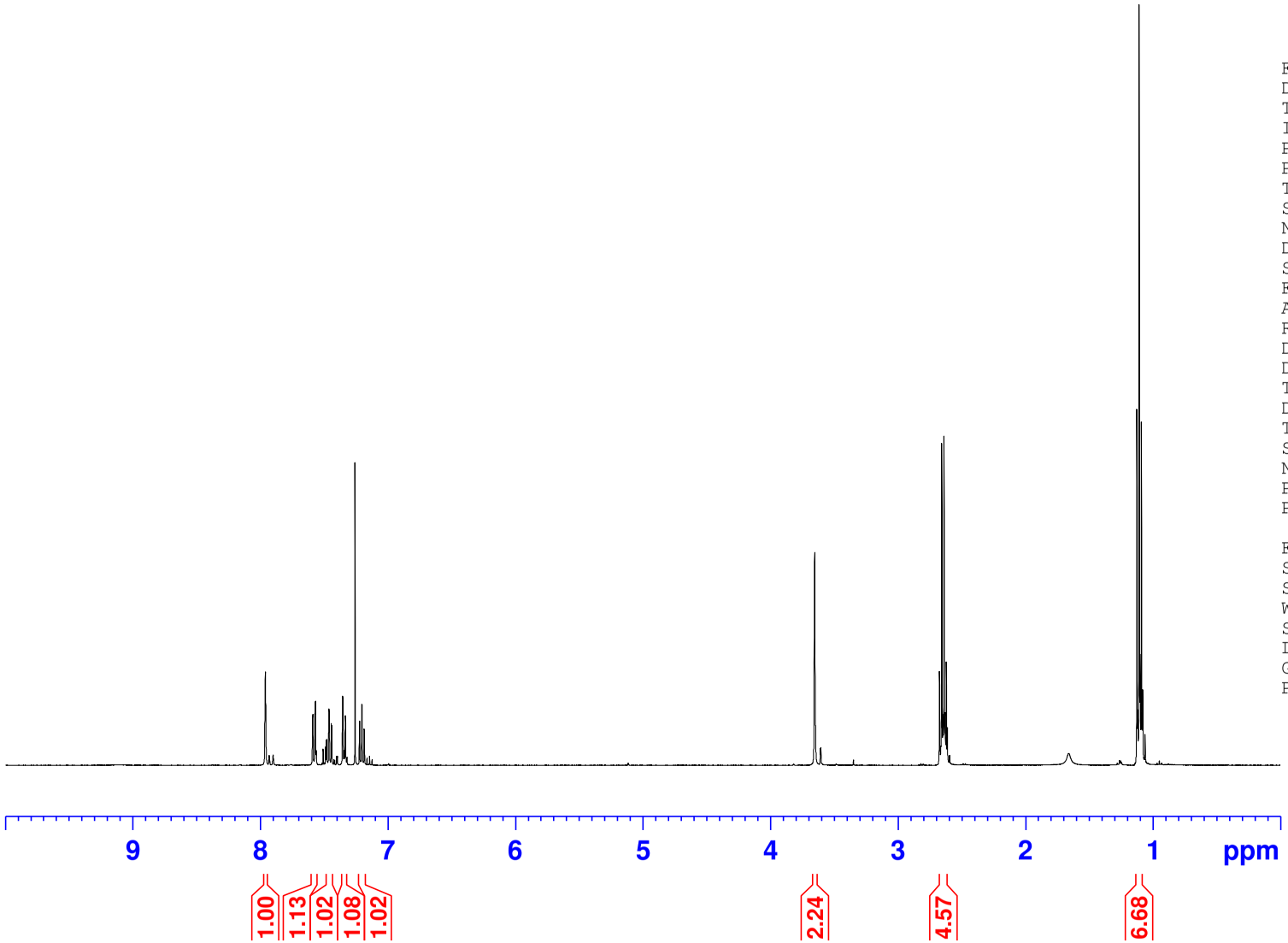
2.675 2.657 2.639 2.621

1.127 1.109 1.091



3.10

170



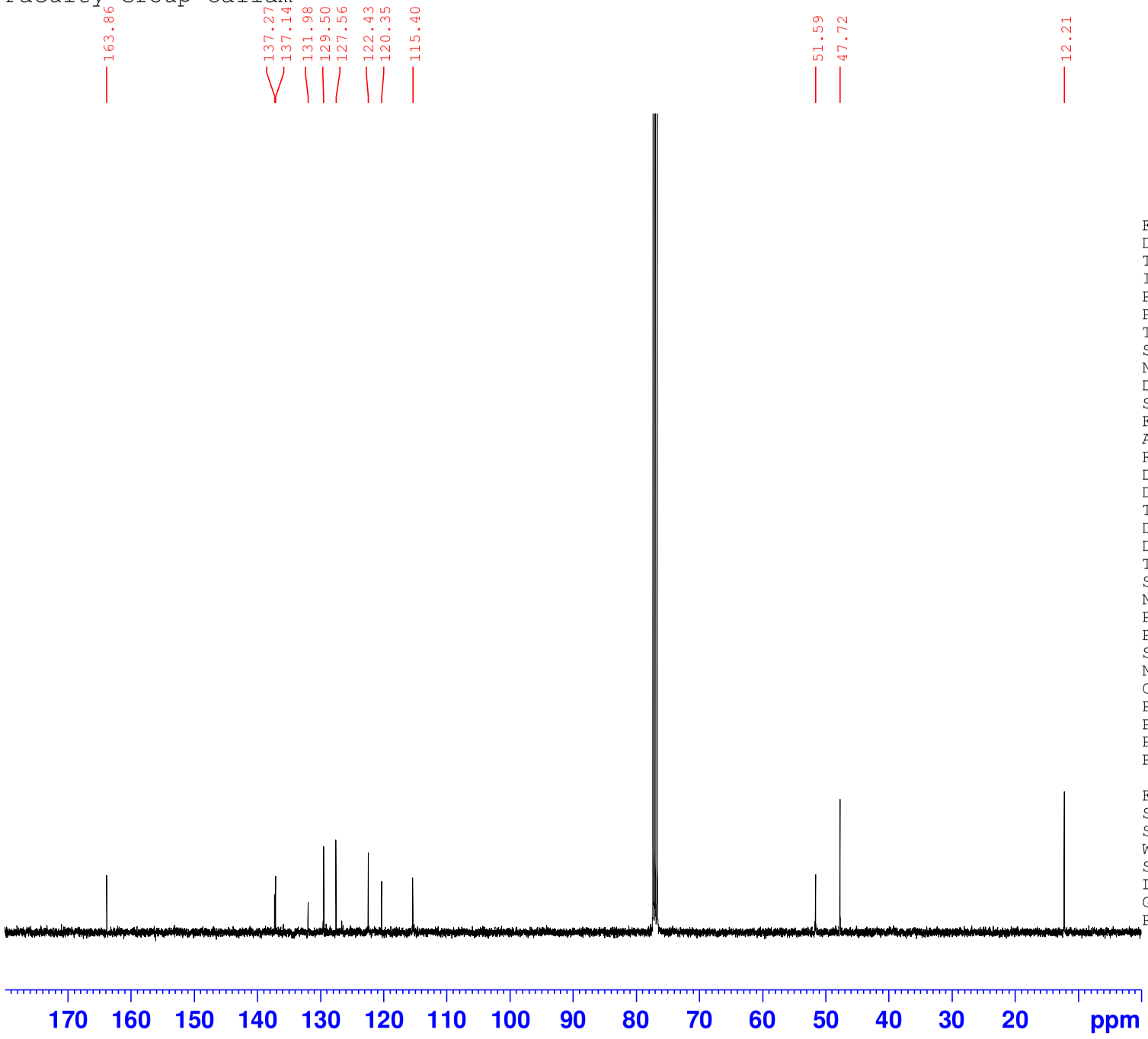
F2 - Acquisition Parameters

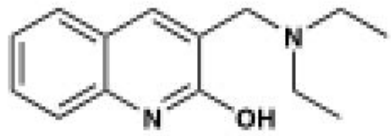
Date_	20180403	
Time	12.56	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700105	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





3.10

F2 - Acquisition Parameters

Date_	20180404
Time	4.42 h
INSTRUM	spect
PROBHD	Z108618_0433 (
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	1024
DS	4
SWH	24038.461 Hz
FIDRES	0.733596 Hz
AQ	1.3631488 sec
RG	2050
DW	20.800 usec
DE	6.50 usec
TE	300.5 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
SFO1	100.6328888 MHz
NUC1	13C
P1	9.50 usec
PLW1	53.29999924 W
SFO2	400.1716007 MHz
NUC2	1H
CPDPRG[2	waltz16
PCPD2	90.00 usec
PLW2	14.60000038 W
PLW12	0.40555999 W
PLW13	0.20399000 W

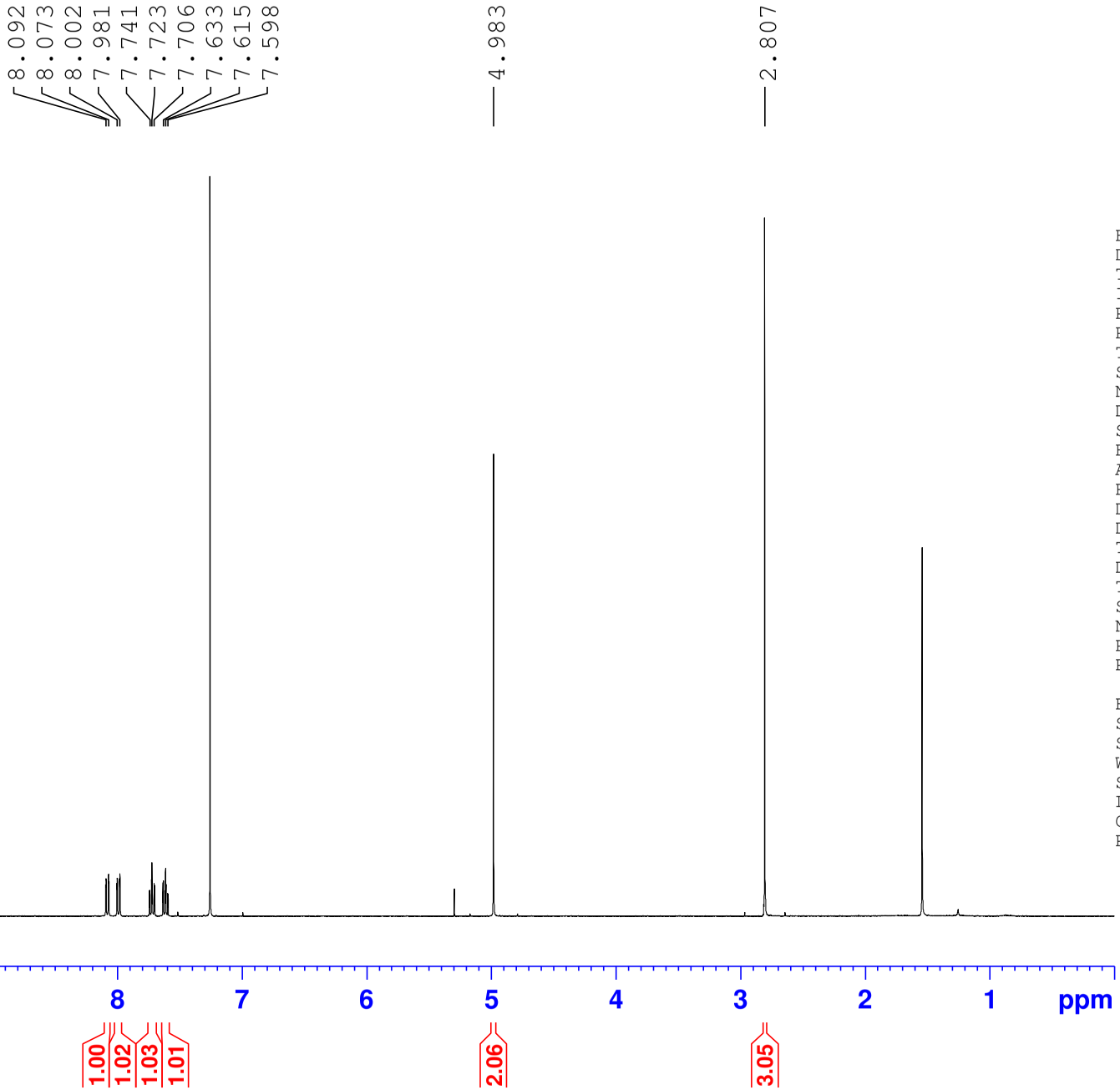
F2 - Processing parameters

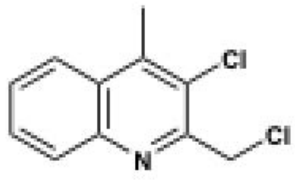
SI	32768
SF	100.6228265 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

APPENDIX C

SELECT ^{1}HNMR AND ^{13}CNMR DATA FROM CHAPTER 4

Faculty Group Callam





4.1

F2 - Acquisition Parameters

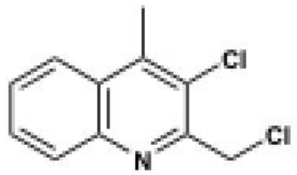
Date_	20180225	
Time	10.42	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

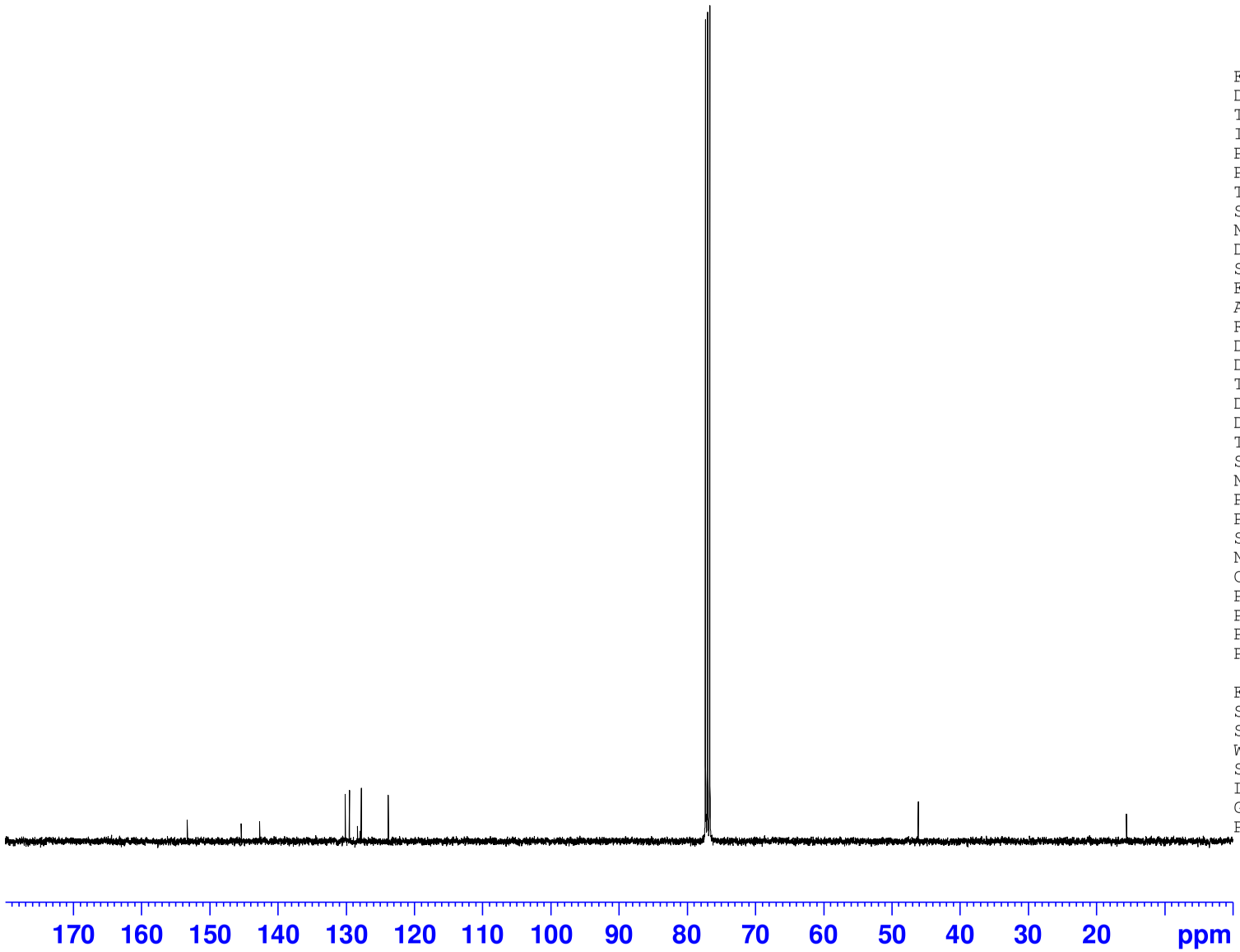
SI	65536	
SF	400.1700108	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

153.31
145.41
142.70
130.16
129.54
128.33
127.97
127.78
123.83
46.11
15.61



4.1

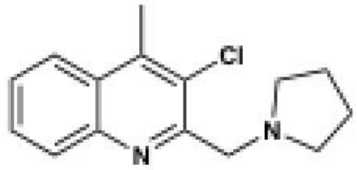


174

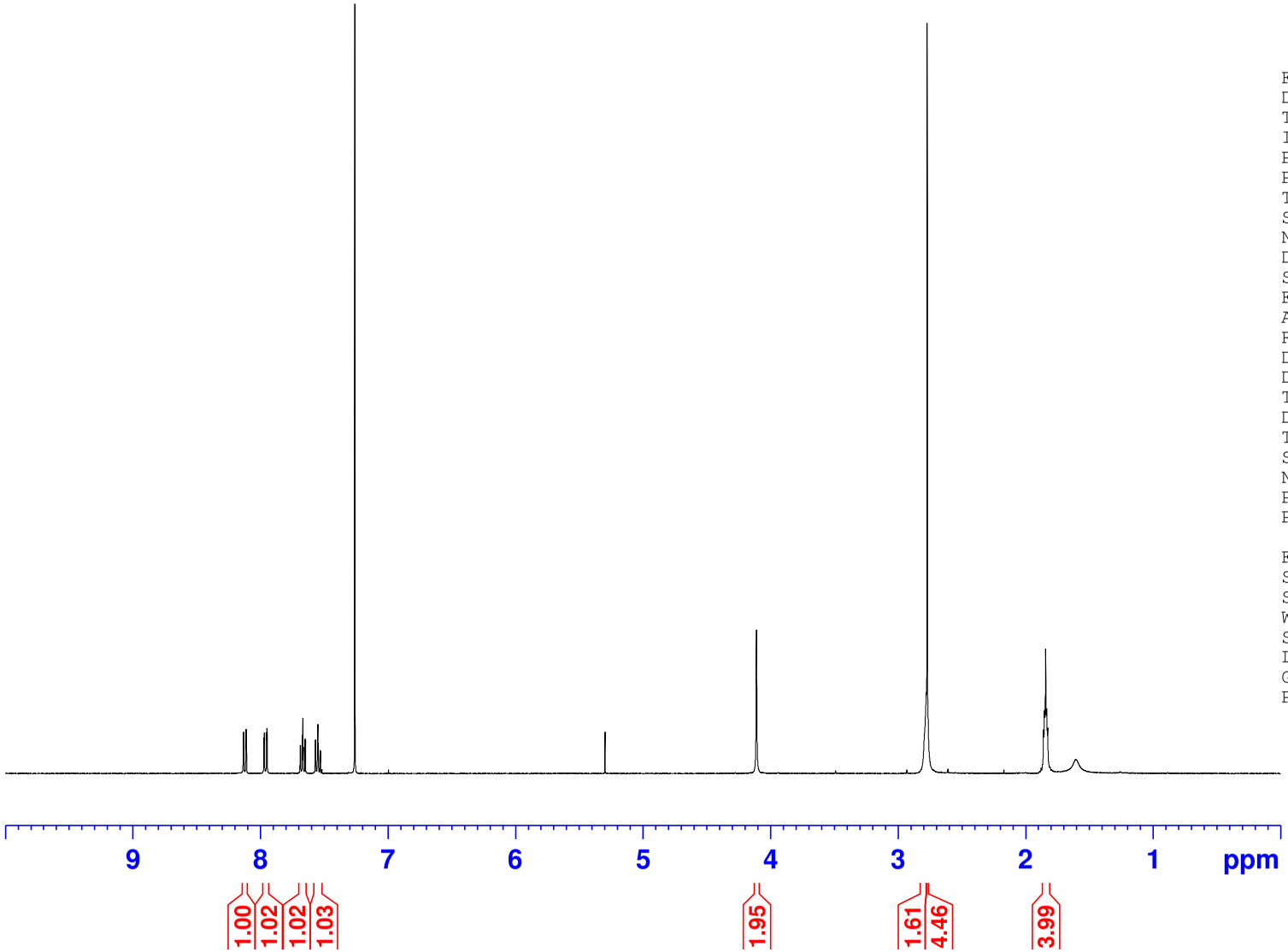
```
F2 - Acquisition Parameters
Date_           20180311
Time              20.48 h
INSTRUM             spect
PROBHD   Z108618_0433 (
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE               300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1                13C
P1                9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2            90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

Faculty Group Callam

8.134
8.132
8.113
8.111
7.970
7.689
7.686
7.672
7.668
7.571
7.567
7.550
4.110
2.778
2.771
1.843



4.2



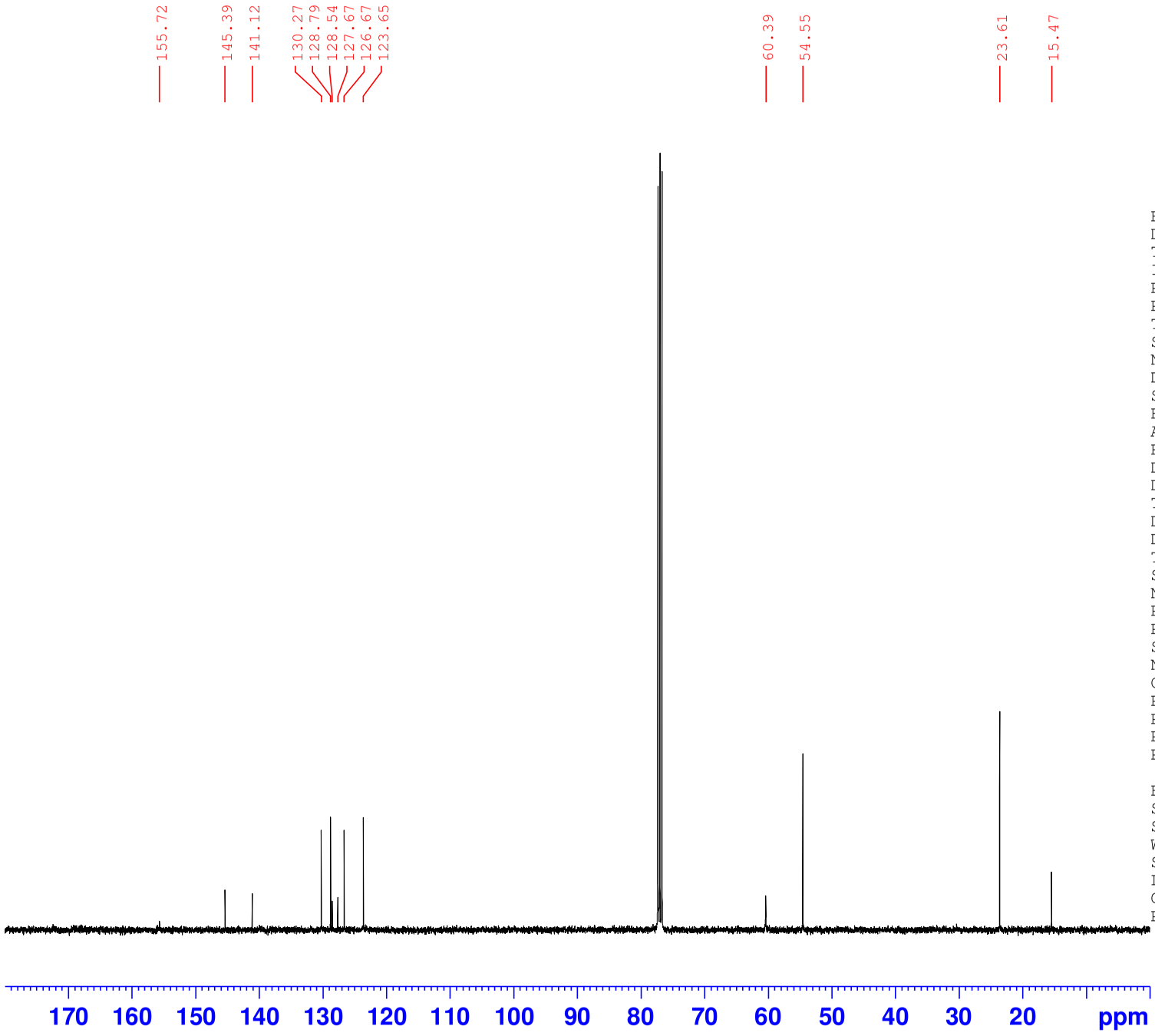
F2 - Acquisition Parameters

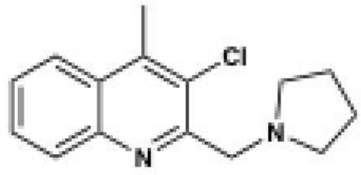
Date_	20180225	
Time	12.07	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700101	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





4.2

F2 - Acquisition Parameters

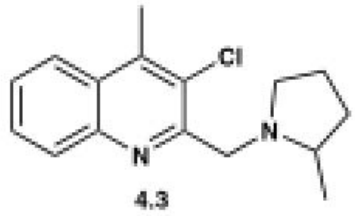
Date_	20180311	
Time	23.09	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

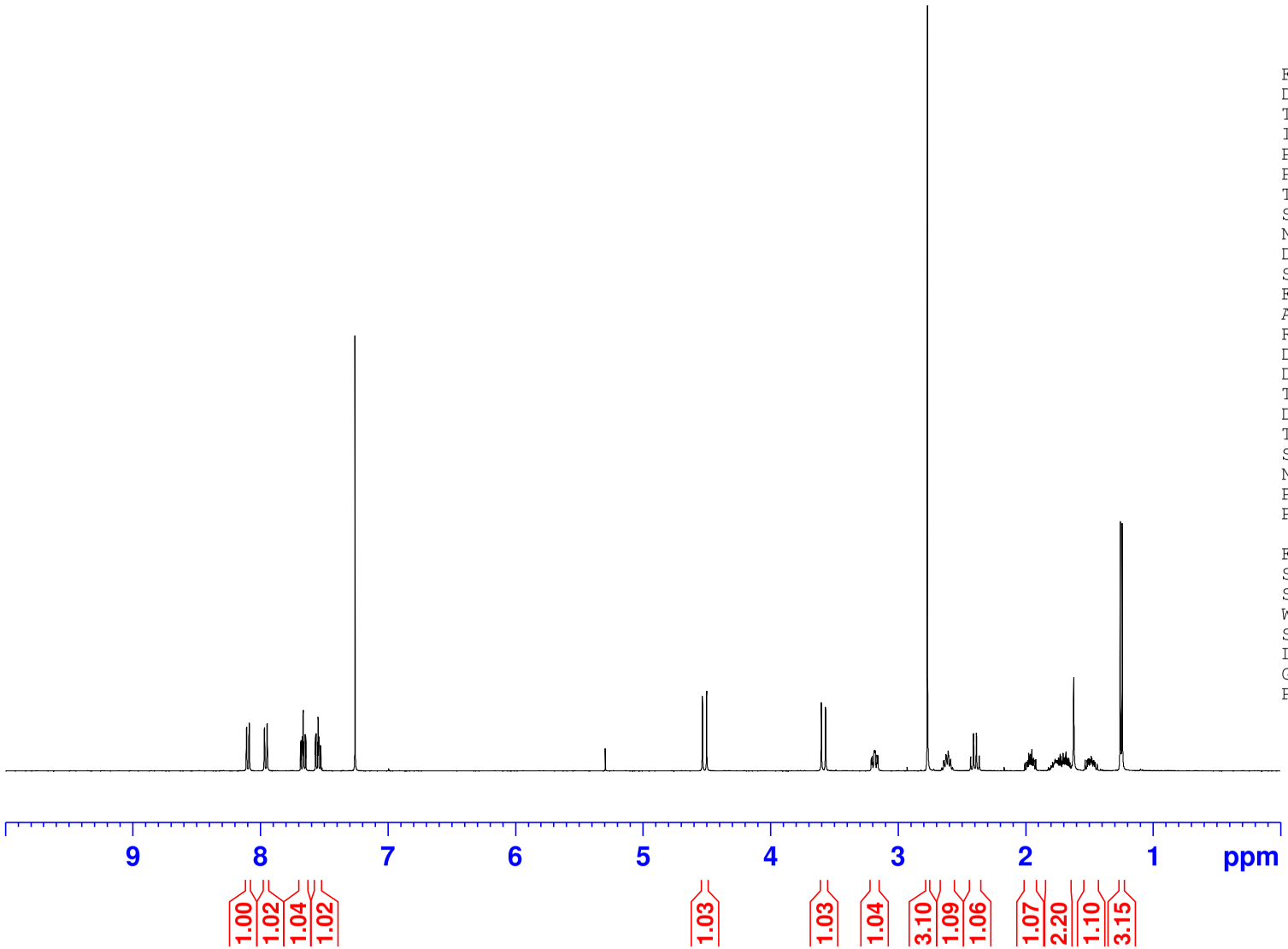
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam

8.110 8.108 8.089 7.968 7.949 7.947 7.683 7.665 7.648 7.565 7.548 7.530 4.534 4.501 3.601 3.568 3.209 3.202 3.186 3.182 3.179 3.165 3.158 2.770 2.624 2.607 2.588 2.430 2.408 2.385 2.363 1.974 1.968 1.962 1.950 1.742 1.728 1.721 1.706 1.496 1.485 1.480 1.257 1.242



4.3



177

F2 - Acquisition Parameters

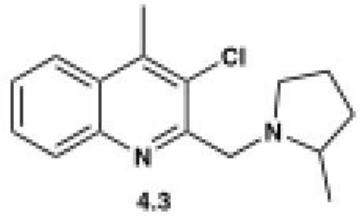
Date_	20180225	
Time	13.24	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

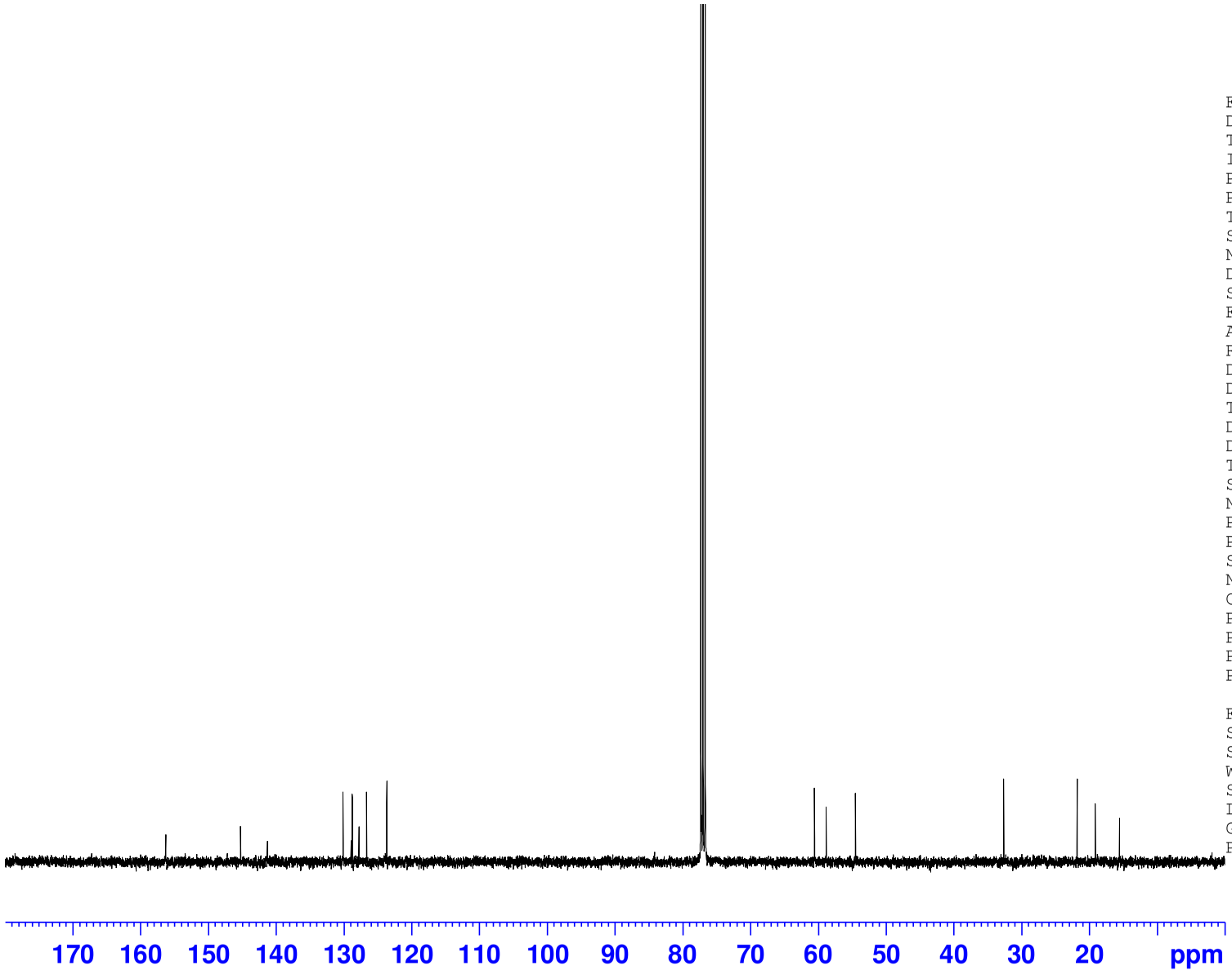
SI	65536	
SF	400.1700106	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

156.29
145.28
141.30
130.14
128.95
128.78
127.77
126.68
123.69
60.57
58.85
54.52
32.64
21.81
19.14
15.54



4.3



178

F2 - Acquisition Parameters

Date_	20180225	
Time	14.24	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

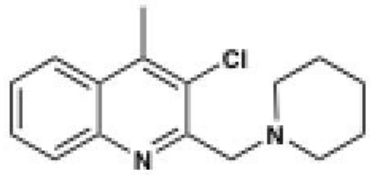
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam

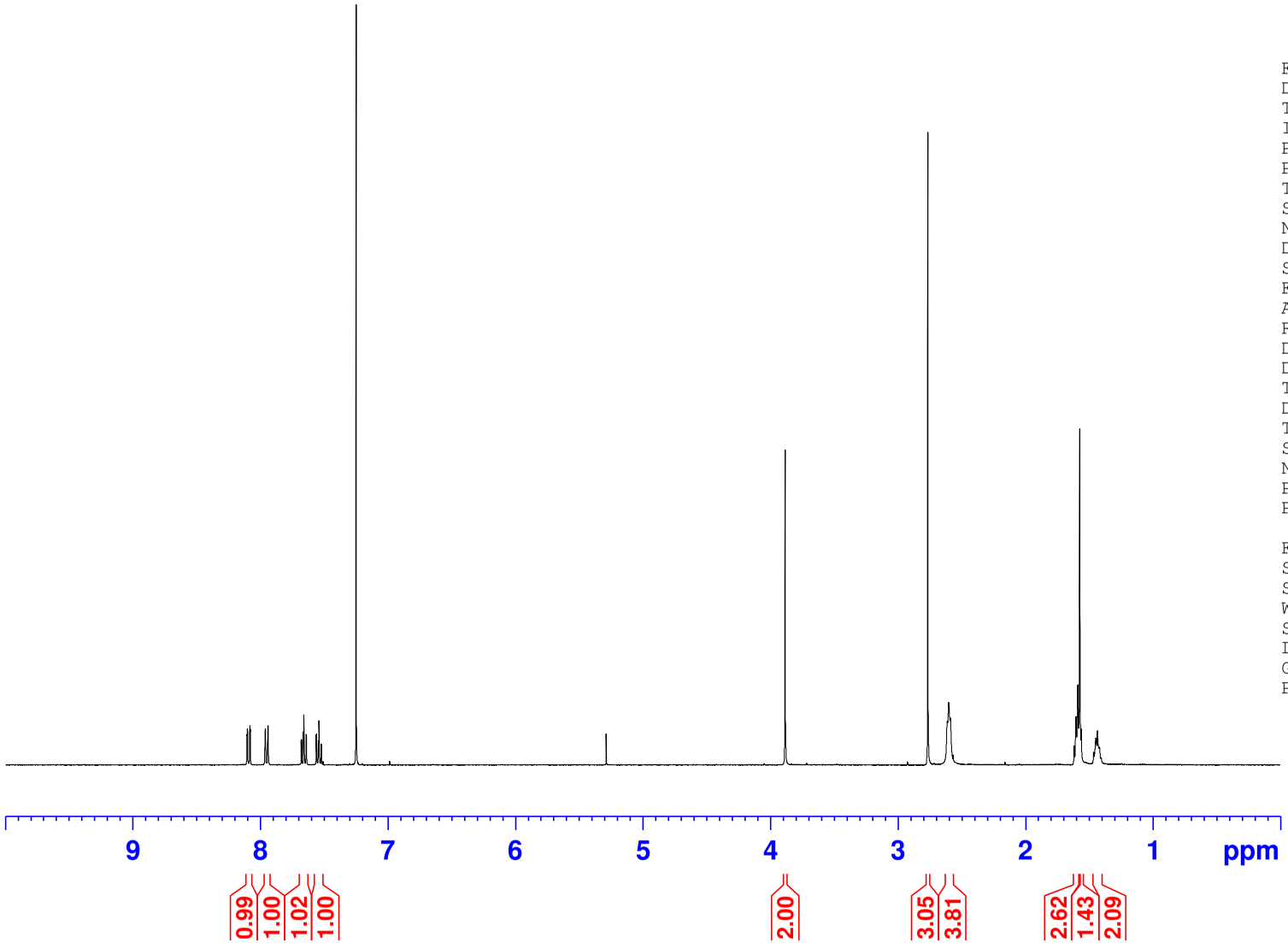
8.104 8.102 8.083 7.961 7.940 7.680 7.677 7.663 7.659 7.642 7.639 7.560 7.542 7.525

3.886

2.767 2.614 2.604 2.590 1.619 1.604 1.590 1.575 1.563 1.465 1.450 1.436 1.422 1.407



4.4



F2 - Acquisition Parameters

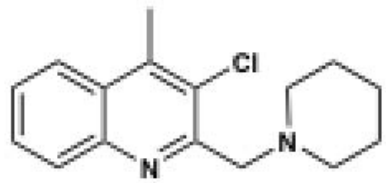
Date_	20180225	
Time	16.14	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

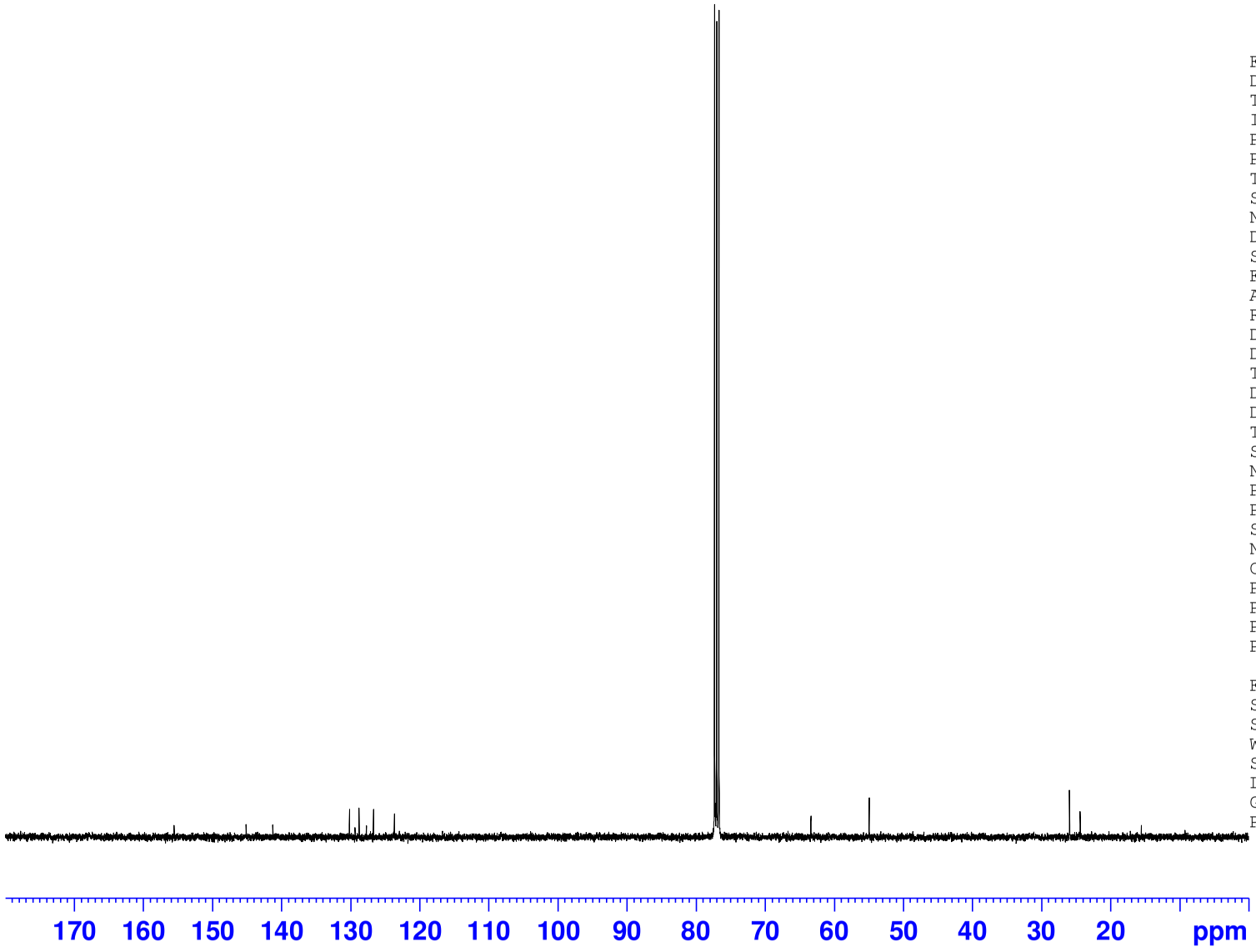
SI	65536	
SF	400.1700136	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

155.58
145.15
141.27
130.19
129.37
128.79
127.71
126.71
123.68
63.38
54.94
25.95
24.39
15.55



4.4




```
F2 - Acquisition Parameters
Date_           20180225
Time              17.34 h
INSTRUM             spect
PROBHD   Z108618_0433 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1                13C
P1                9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2            90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

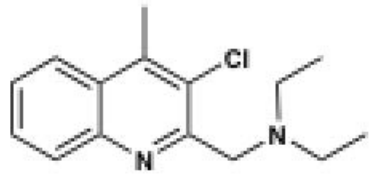
Faculty Group Callam

8.109
8.089
8.088
7.973
7.971
7.953
7.952
7.950
7.948
7.688
7.685
7.668
7.650
7.571
7.550
7.529

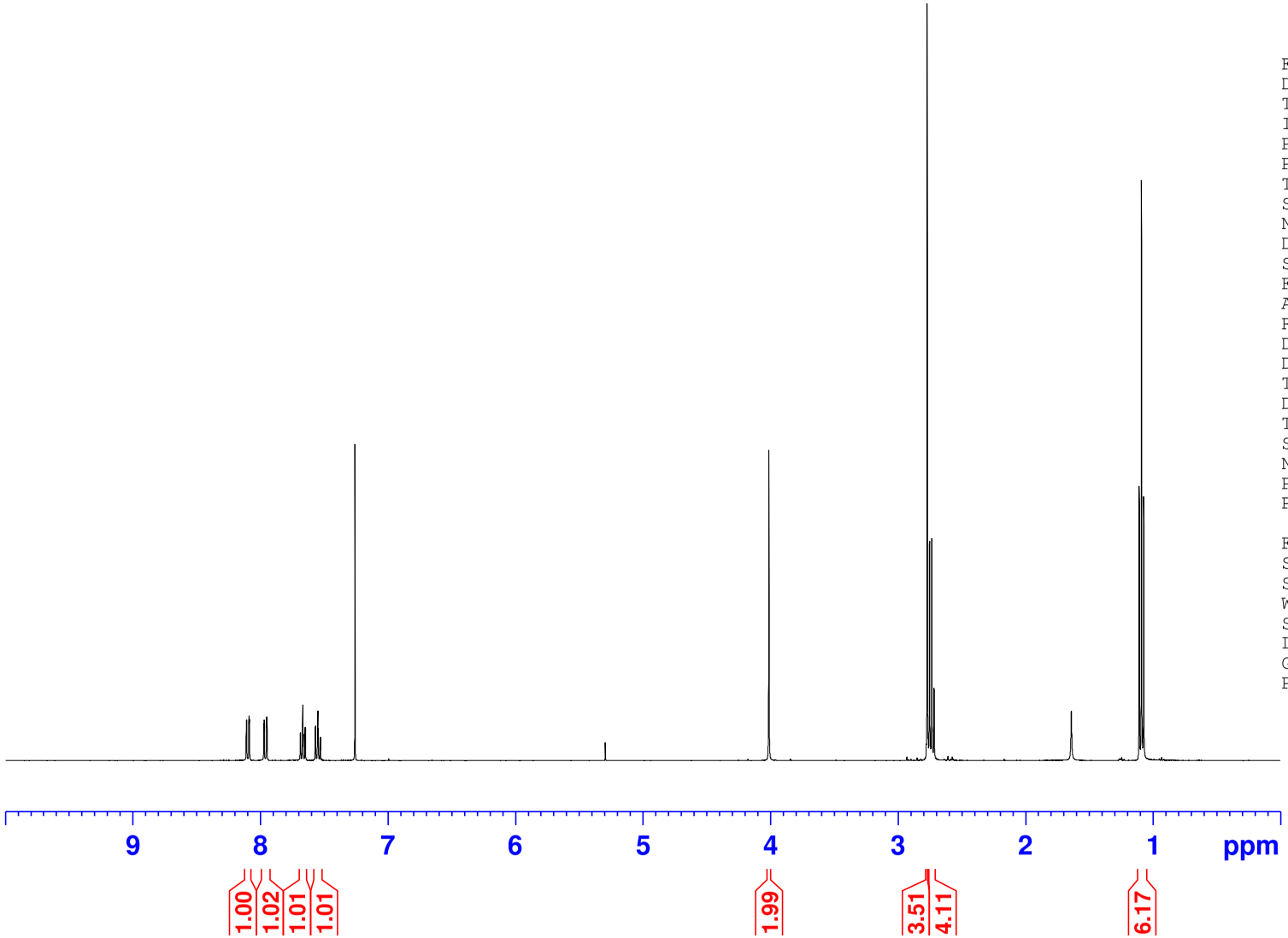
4.013

2.771
2.753
2.735
2.717

1.109
1.091
1.073



4.5



F2 - Acquisition Parameters

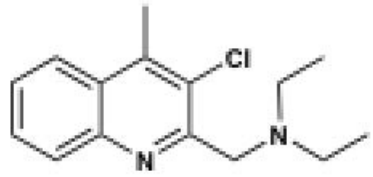
Date_	20180225	
Time	17.39	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700105	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

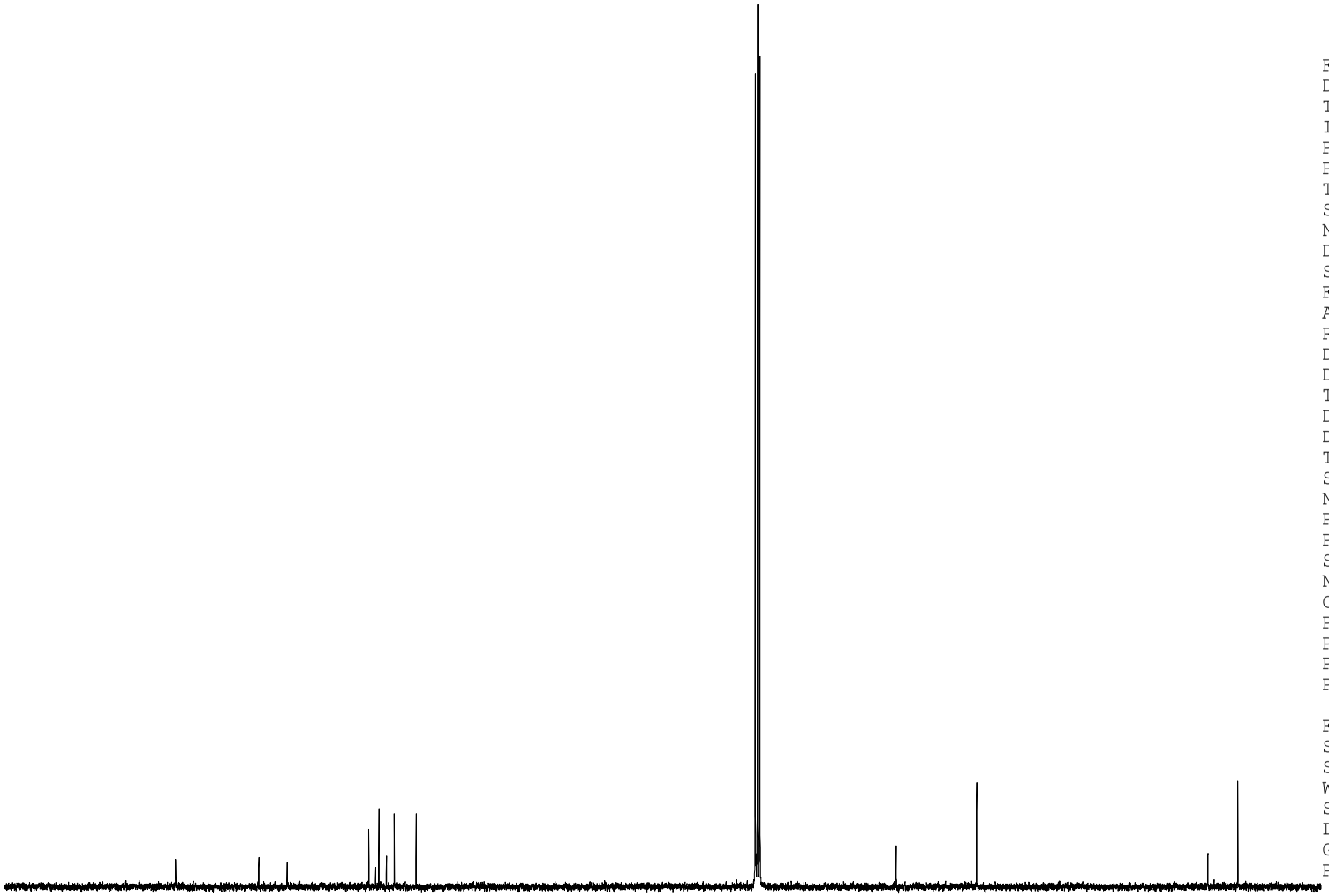
Faculty Group Callam

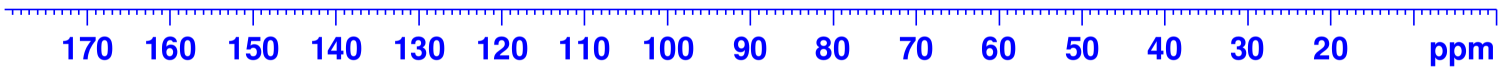
156.54
145.18
141.30
130.14
129.22
128.77
127.74
126.67
123.69
58.09
47.10
15.50
11.40



4.5

182





F2 - Acquisition Parameters

Date_	20180225	
Time	18.39	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

APPENDIX D

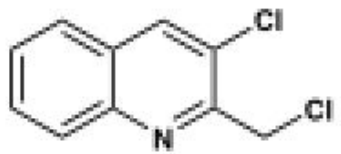
SELECT ^{1}HNMR, ^{13}CNMR, AND HMQC DATA FROM CHAPTER 5

Faculty Group Callam

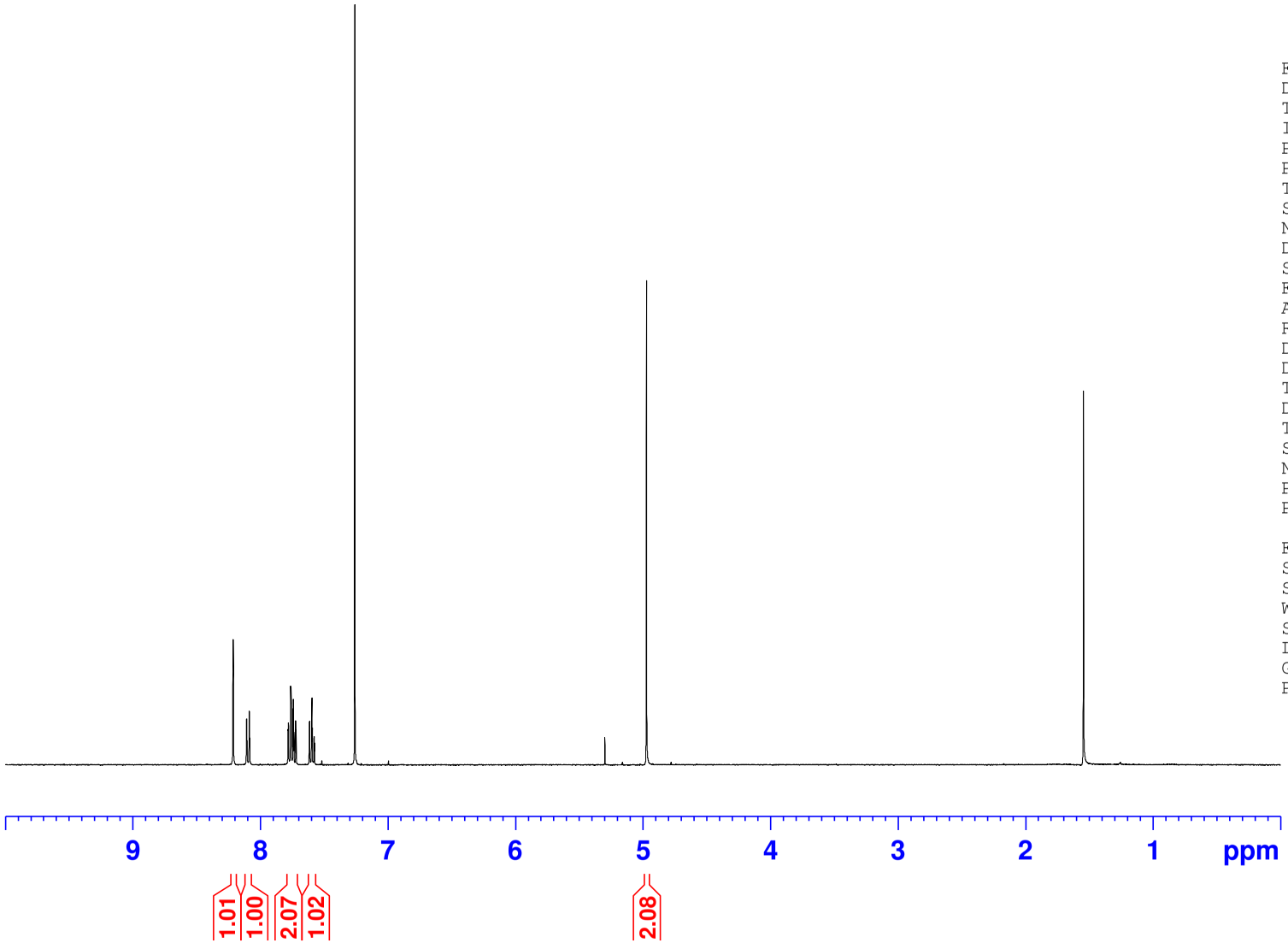
8.214
8.109
8.106
8.088
8.085
7.783
7.781
7.780
7.762
7.759
7.741
7.724
7.617
7.614
7.597
7.579
7.577

4.973

1.546



5.1



184

F2 - Acquisition Parameters

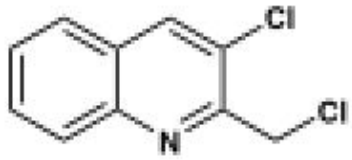
Date_	20180225	
Time	18.45	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700101	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

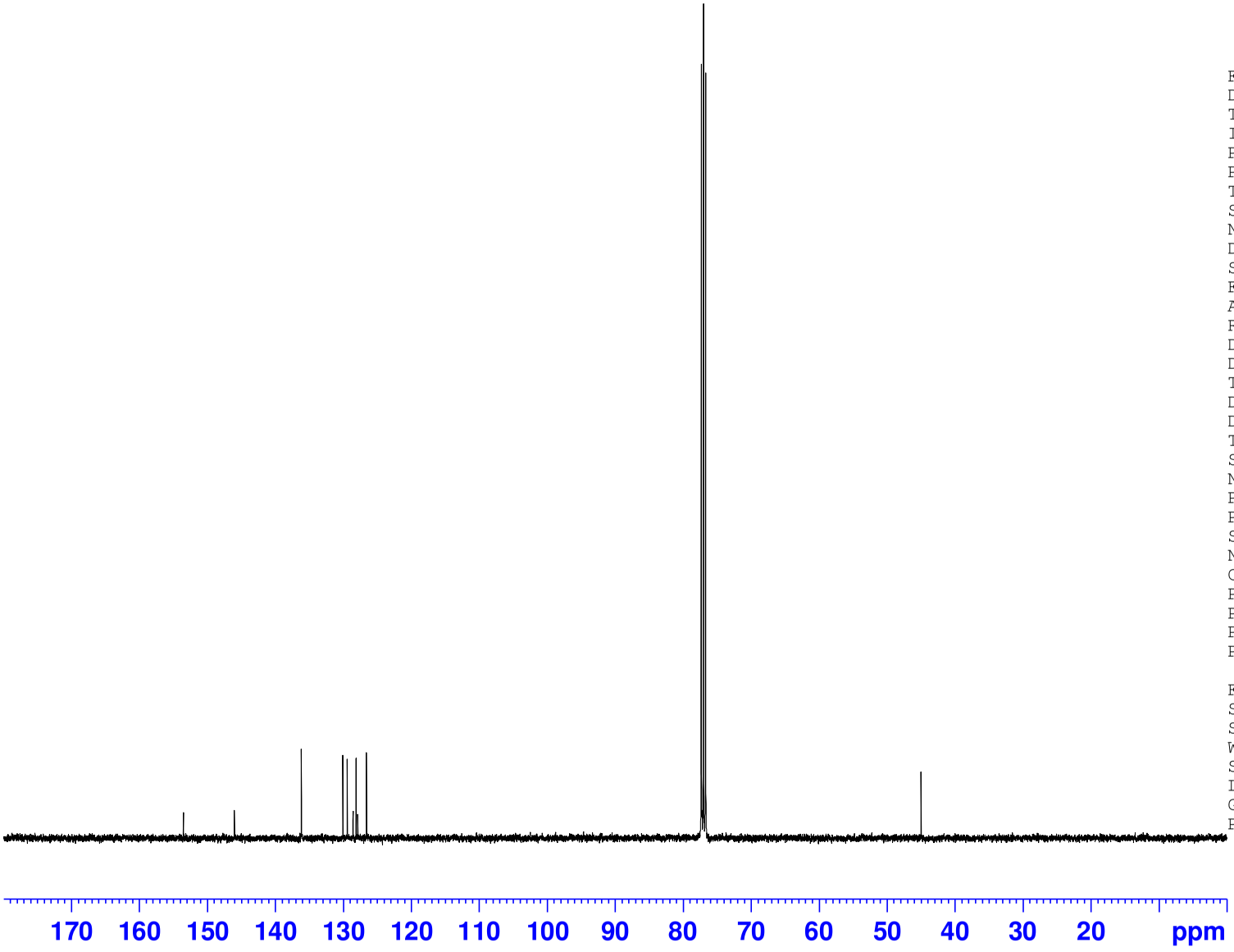
Faculty Group Callam

153.52
146.05
136.18
130.10
129.44
128.58
128.13
127.89
126.62
45.01



5.1

185




```
F2 - Acquisition Parameters
Date_           20180312
Time                0.12 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ             1.3631488 sec
RG                  2050
DW                20.800 usec
DE                 6.50 usec
TE                300.5 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                 32768
SF           100.6228265 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

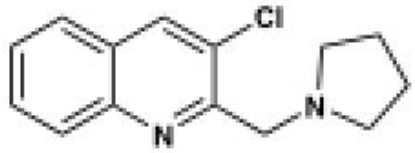
Faculty Group Callam

8.140
8.138
8.116
7.726
7.706
7.696
7.675
7.658
7.541
7.538
7.520
7.503
7.501

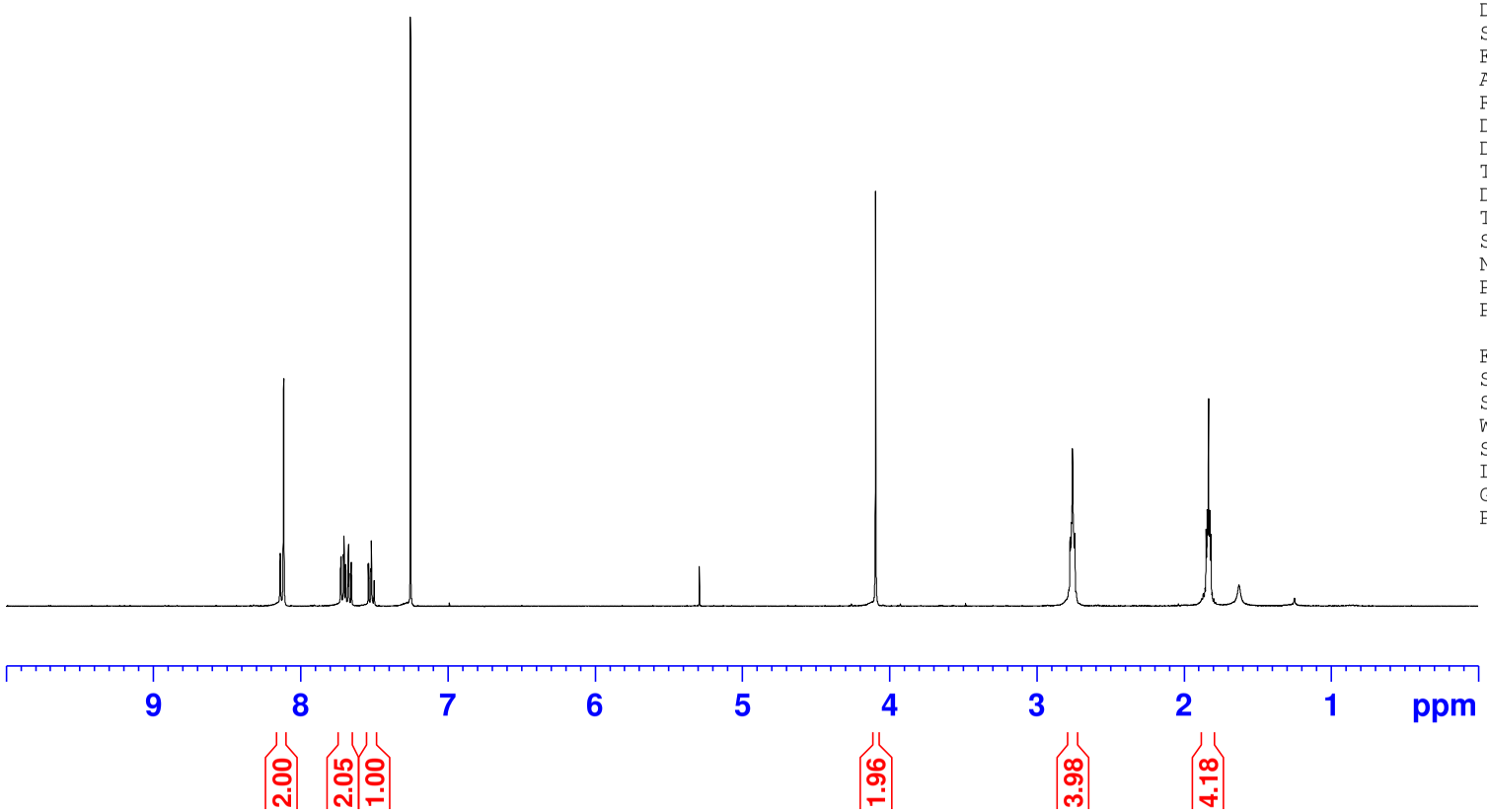
4.095

2.757

1.833



5.2



186

F2 - Acquisition Parameters

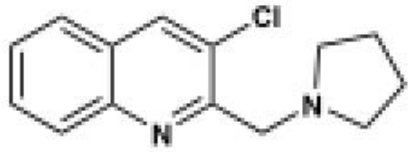
Date_	20180208	
Time	4.30	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

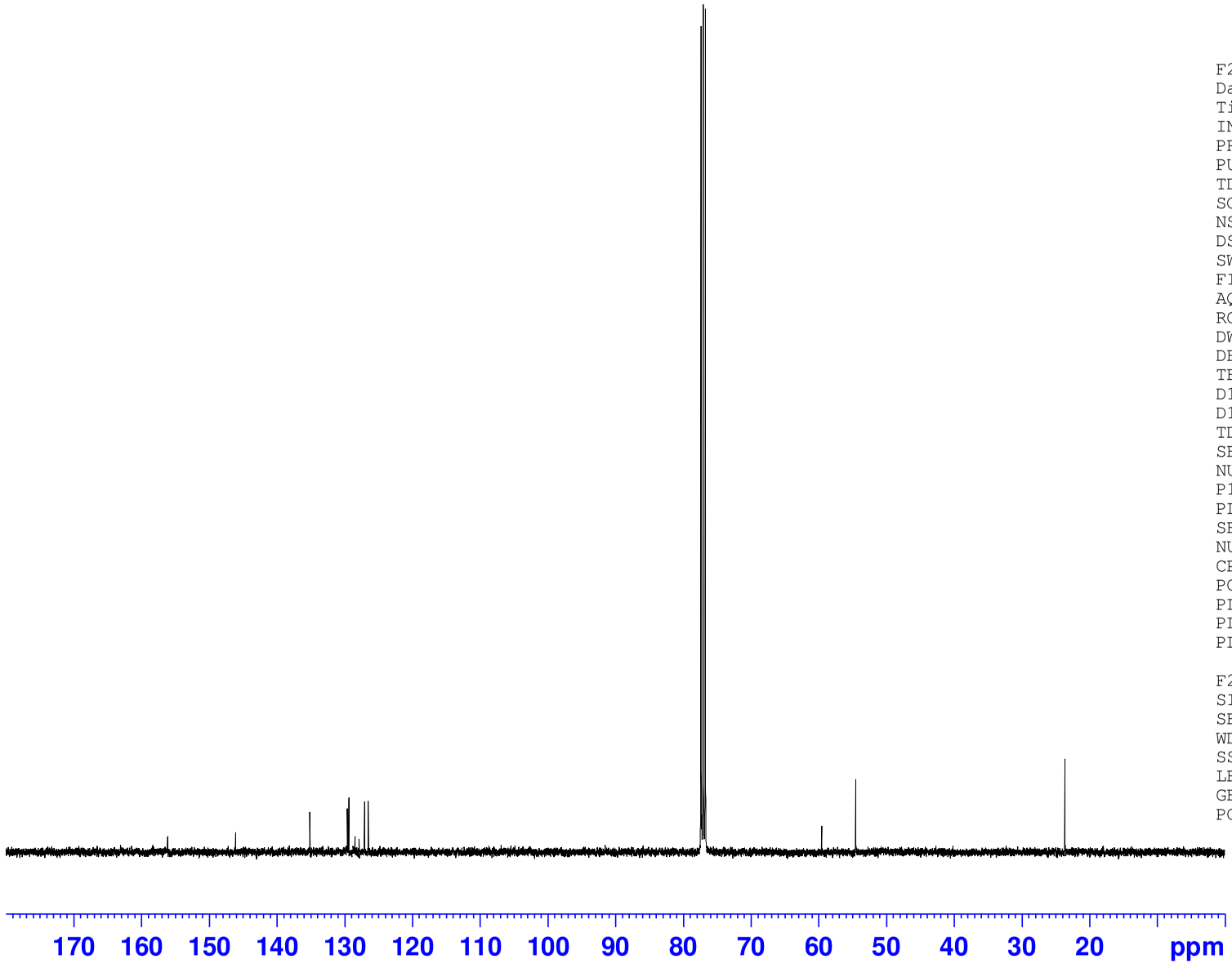
SI	65536	
SF	400.1700124	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

156.10
146.07
135.10
129.55
129.34
128.45
127.83
127.02
126.47
59.50
54.49
23.60

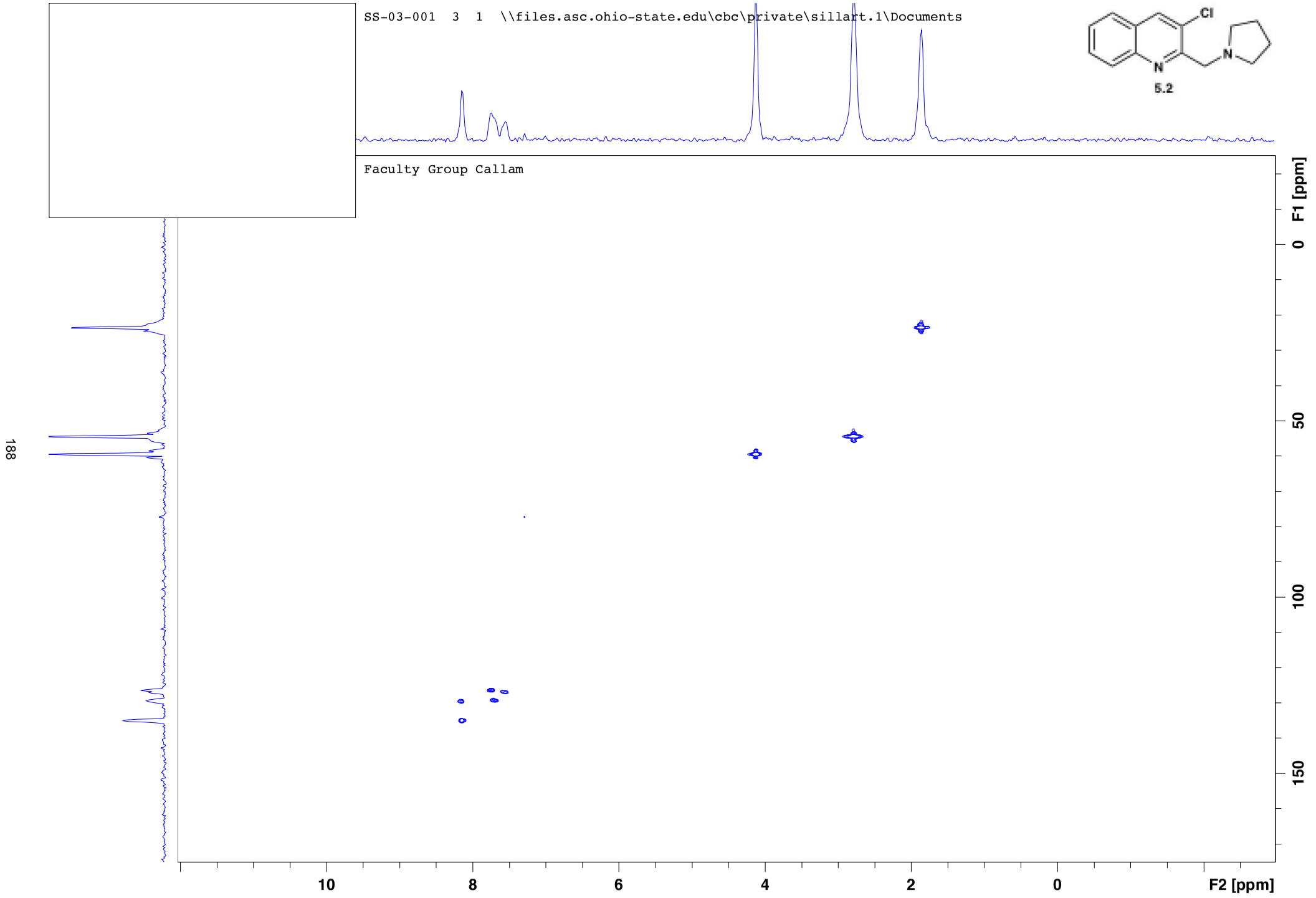


5.2



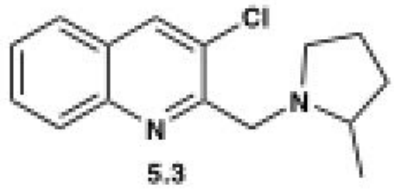
187

```
F2 - Acquisition Parameters
Date_           20180208
Time                5.30 h
INSTRUM             spect
PROBHD    Z108618_0433 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.733596 Hz
AQ             1.3631488 sec
RG                  2050
DW                20.800 usec
DE                 6.50 usec
TE                300.8 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                 32768
SF           100.6228265 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

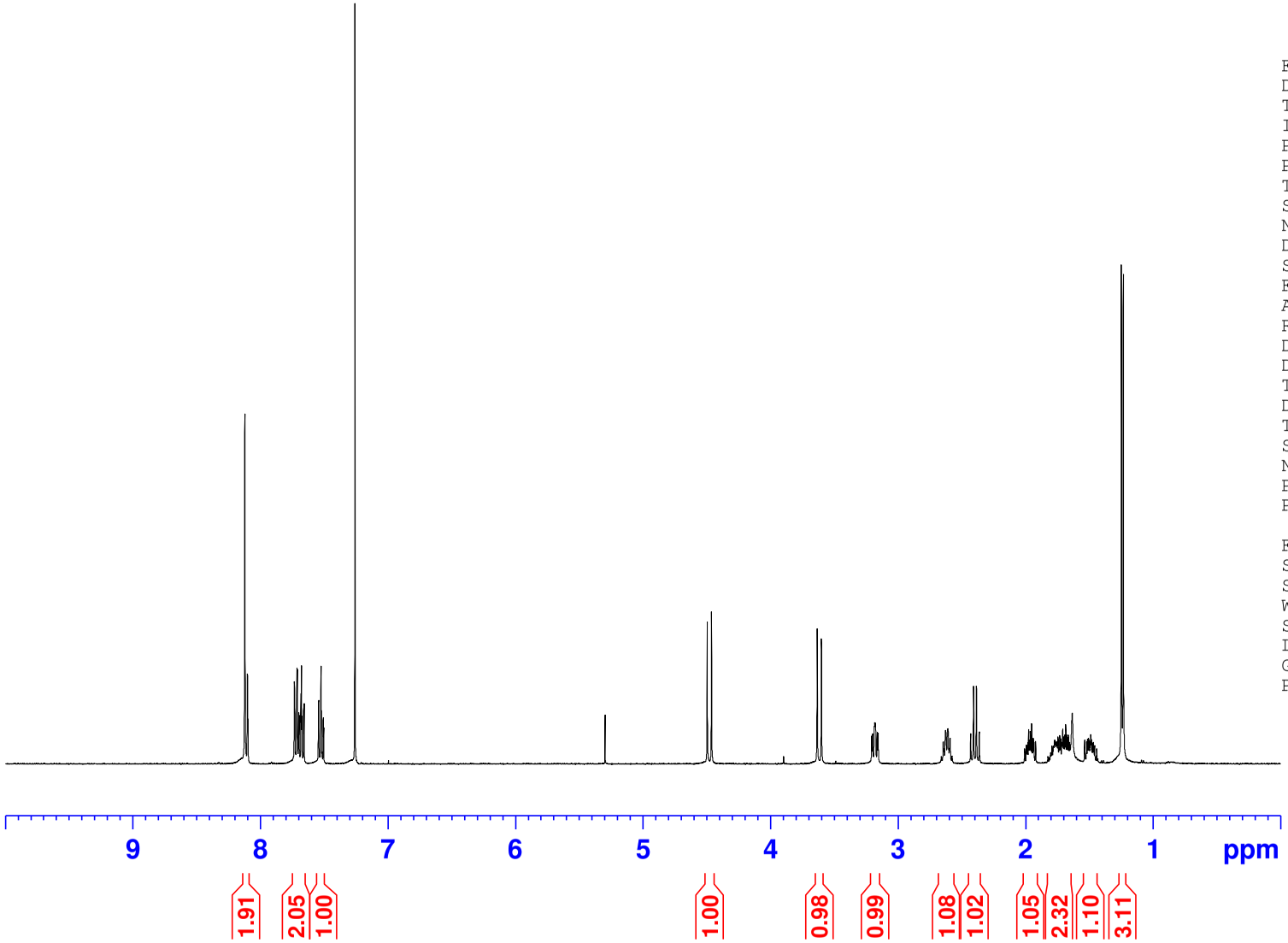

SS-03-001 3 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Cl
N
N
5.2
Faculty Group Callam
188
F1 [ppm]
0
50
100
150
10
8
6
4
2
0
F2 [ppm]

Faculty Group Callam

8.123 8.102 7.735 7.731 7.714 7.711 7.700 7.696 7.682 7.679 7.658 7.544 7.525 7.508 4.496 4.463 3.634 3.601 3.207 3.199 3.183 3.179 3.163 3.156 2.628 2.609 2.430 2.408 2.385 2.363 1.976 1.964 1.952 1.731 1.708 1.699 1.692 1.685 1.677 1.670 1.663 1.489 1.248 1.233



5.3



189

F2 - Acquisition Parameters

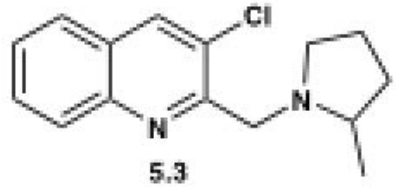
Date_	20180208	
Time	5.36	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

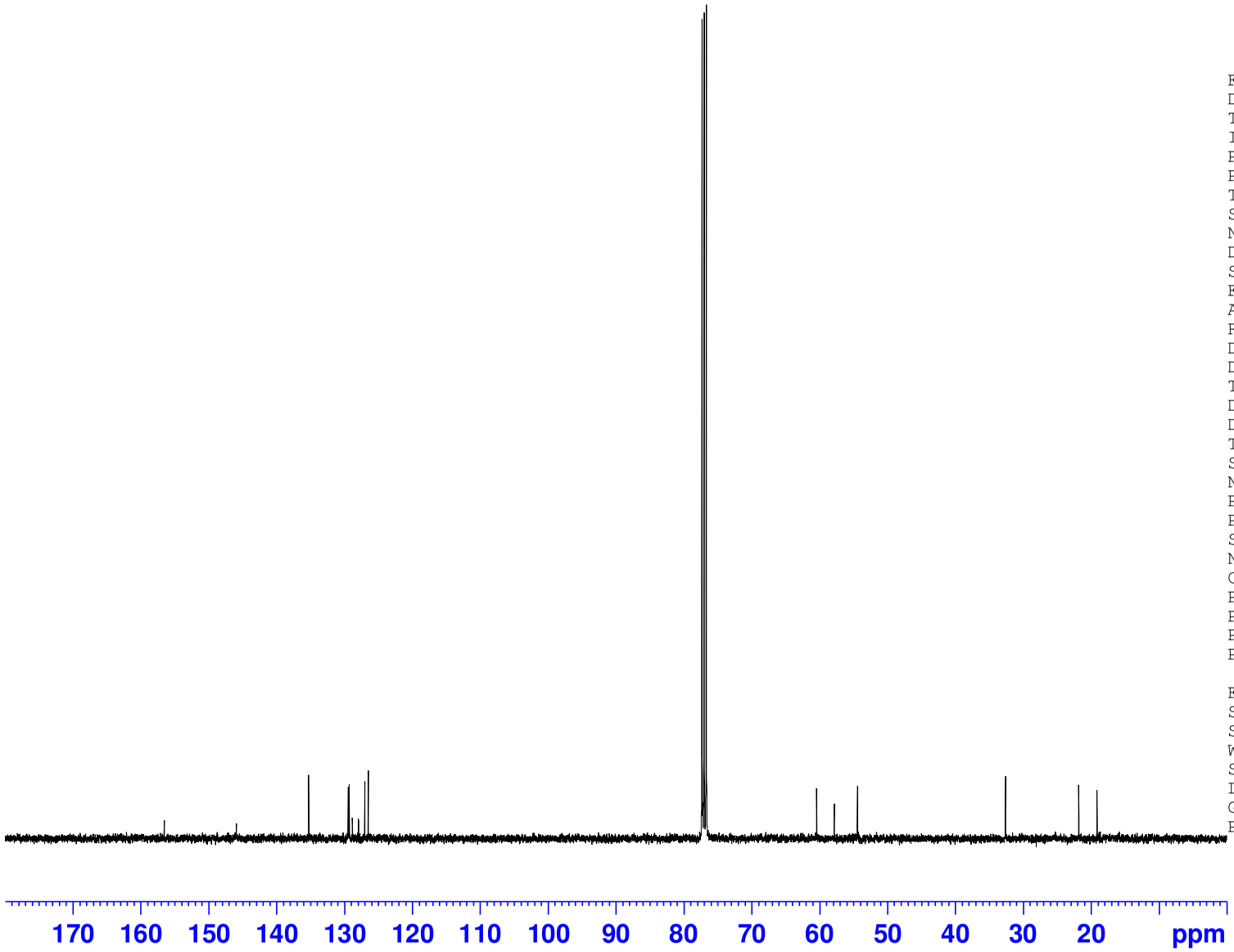
SI	65536	
SF	400.1700103	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

156.57
145.95
135.29
129.45
129.32
128.85
127.94
127.02
126.49
60.45
57.84
54.43
32.62
21.82
19.14



5.3



F2 - Acquisition Parameters

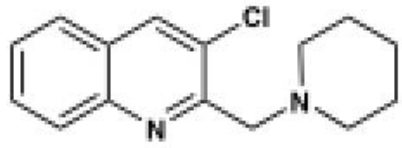
Date_	20180208	
Time	6.36	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.9	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

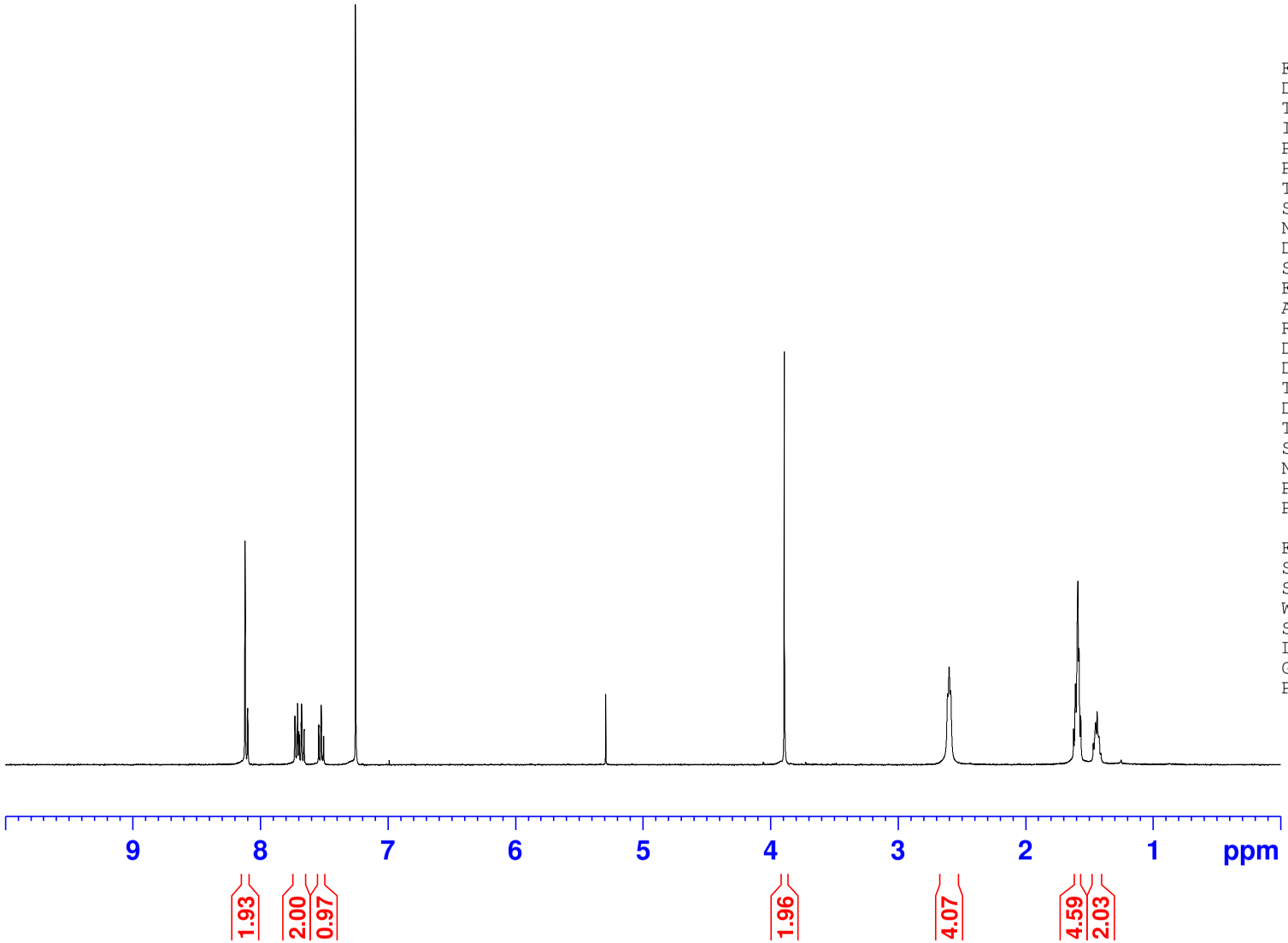
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

Faculty Group Callam

8.121
8.101
8.099
7.728
7.711
7.699
7.677
7.660
7.542
7.524
7.507
3.892
2.600
1.589
1.439



5.4



191

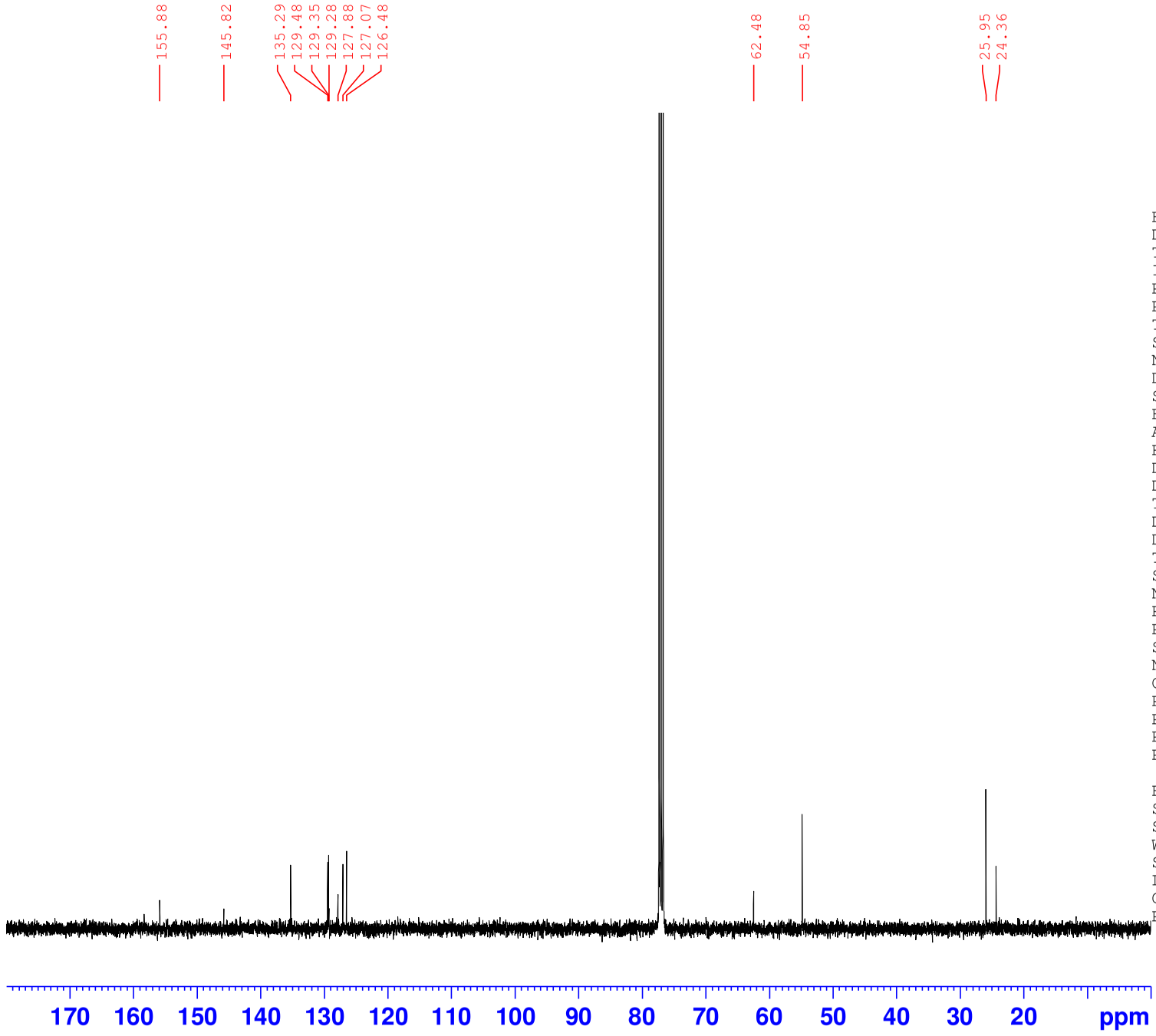
F2 - Acquisition Parameters

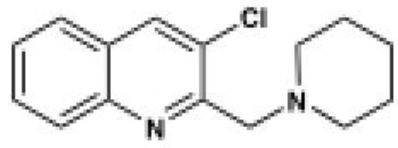
Date_	20180207	
Time	23.08	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700124	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





5.4

F2 - Acquisition Parameters

Date_	20180209	
Time	0.05	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.6	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

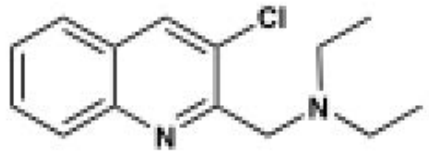
Faculty Group Callam

8.117 8.096 8.094 7.731 7.728 7.711 7.707 7.697 7.693 7.676 7.655 7.541 7.522 7.505 7.502

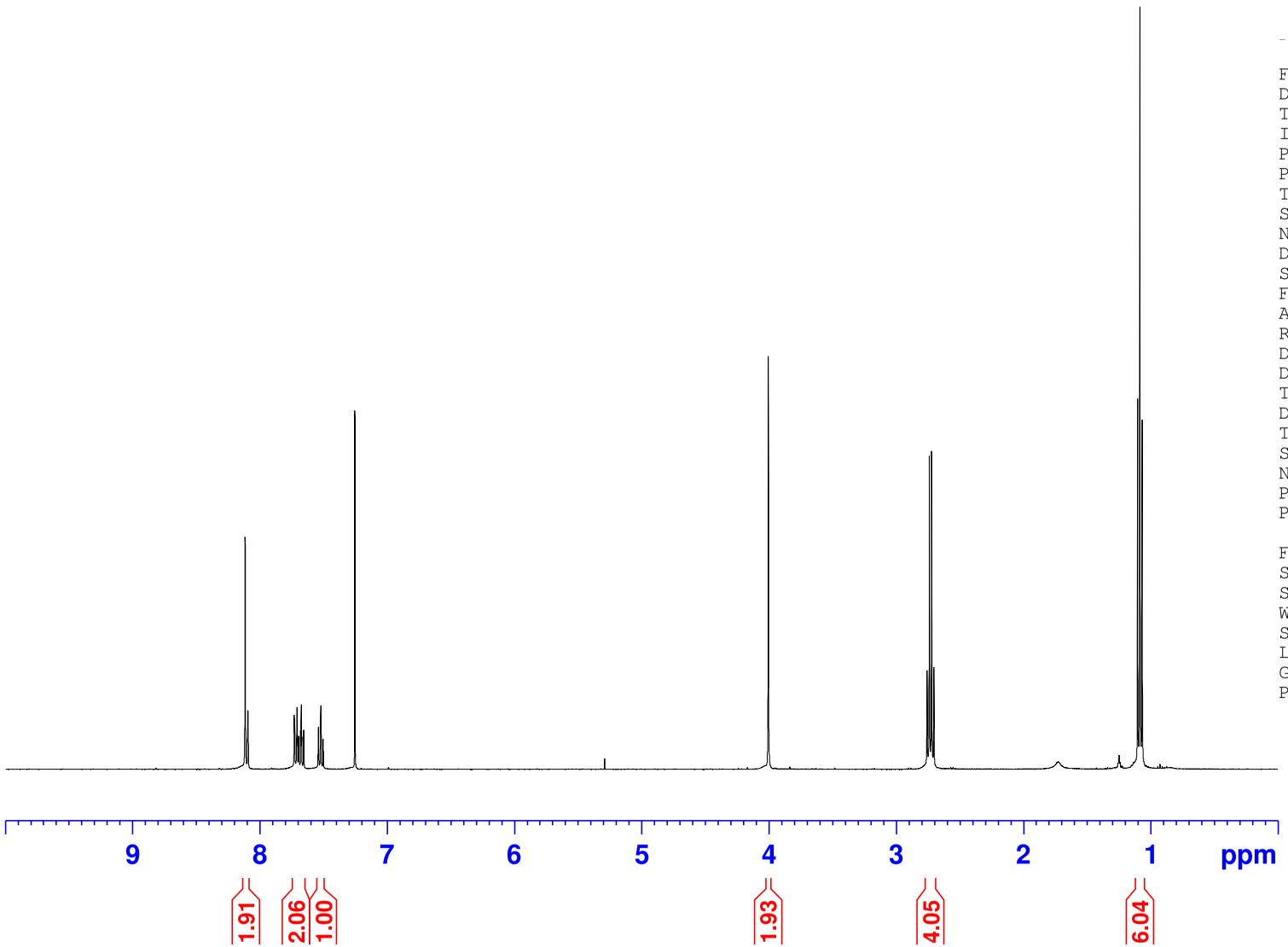
4.005

2.758 2.740 2.722 2.705

1.103 1.086 1.068



5.5



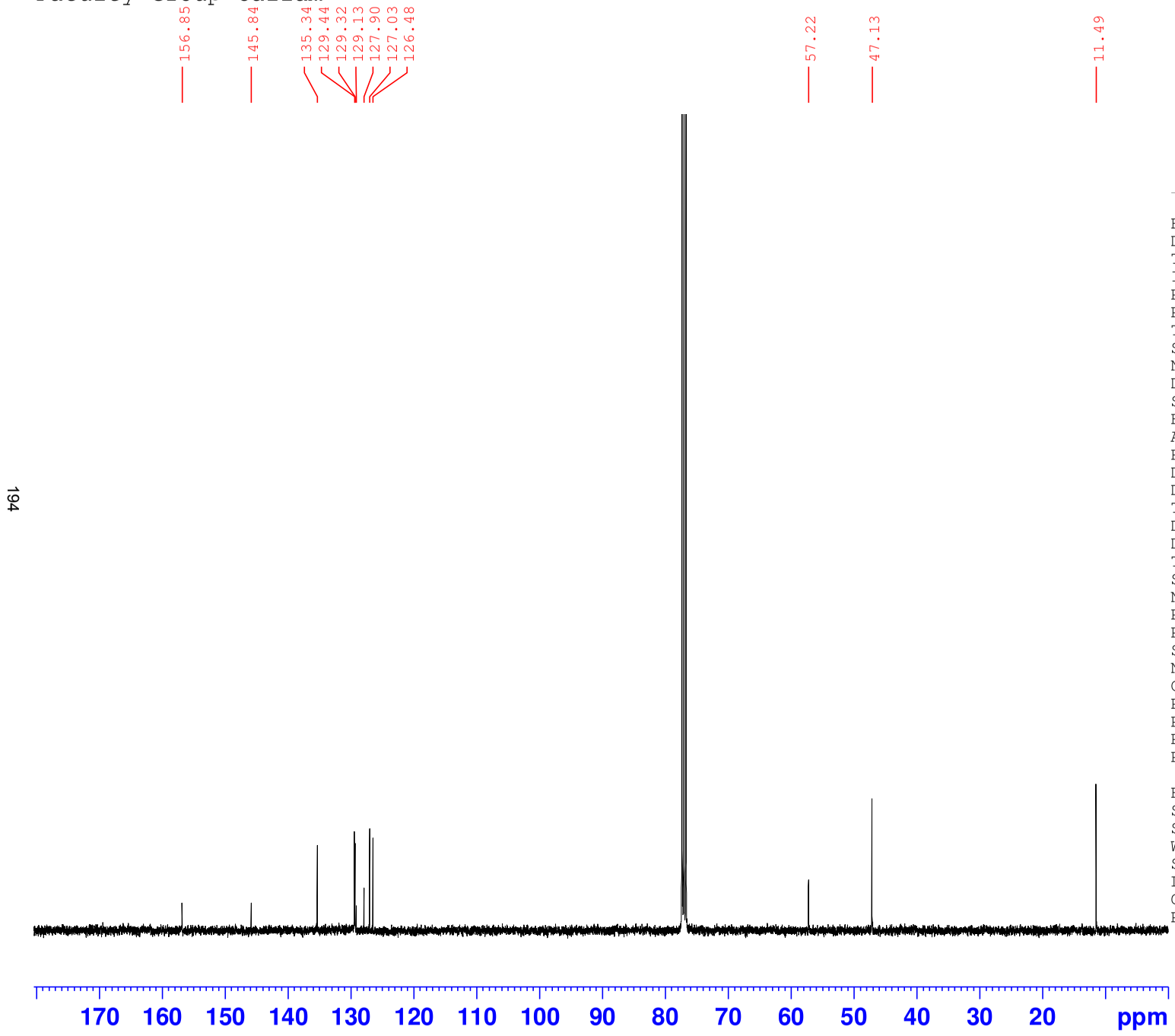
F2 - Acquisition Parameters

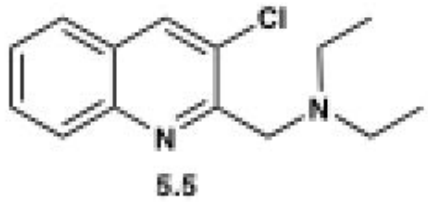
Date_	20180207	
Time	23.13	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700124	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





5.5

F2 - Acquisition Parameters

Date_	20180208	
Time	23.02	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.6	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

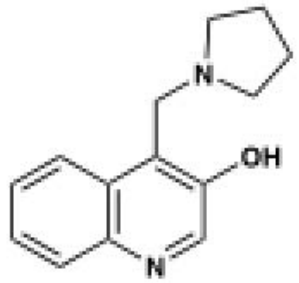
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

APPENDIX E

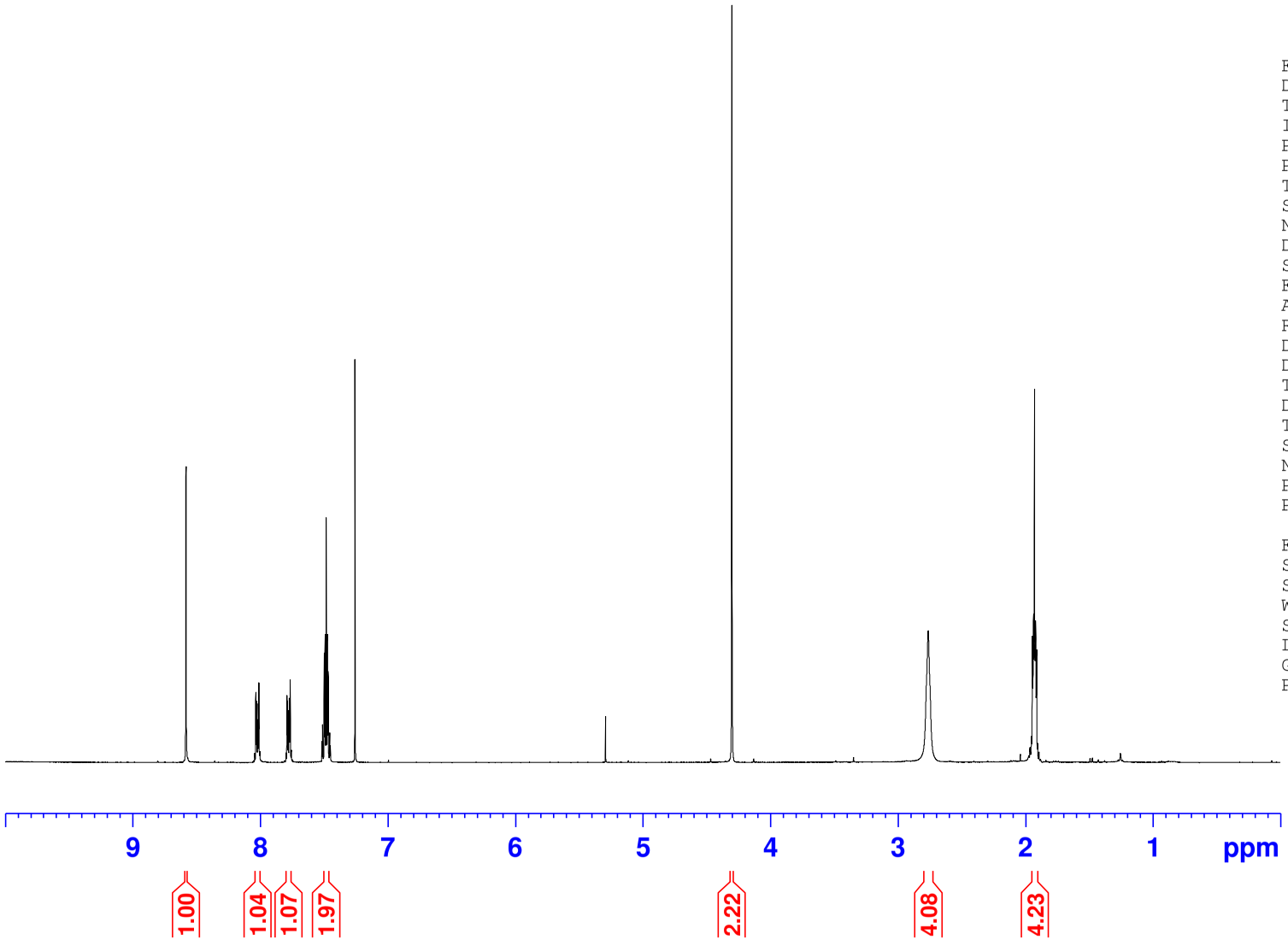
SELECT ^{1}HNMR, ^{13}CNMR, AND HMQC DATA FROM CHAPTER 6

Faculty Group Callam

8.584
8.029
8.022
7.787
7.782
7.773
7.485
4.303
2.763
1.930



6.1



196

F2 - Acquisition Parameters

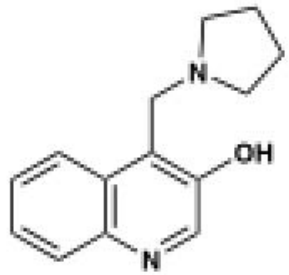
Date_	20180114	
Time	16.57	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.9	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700100	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

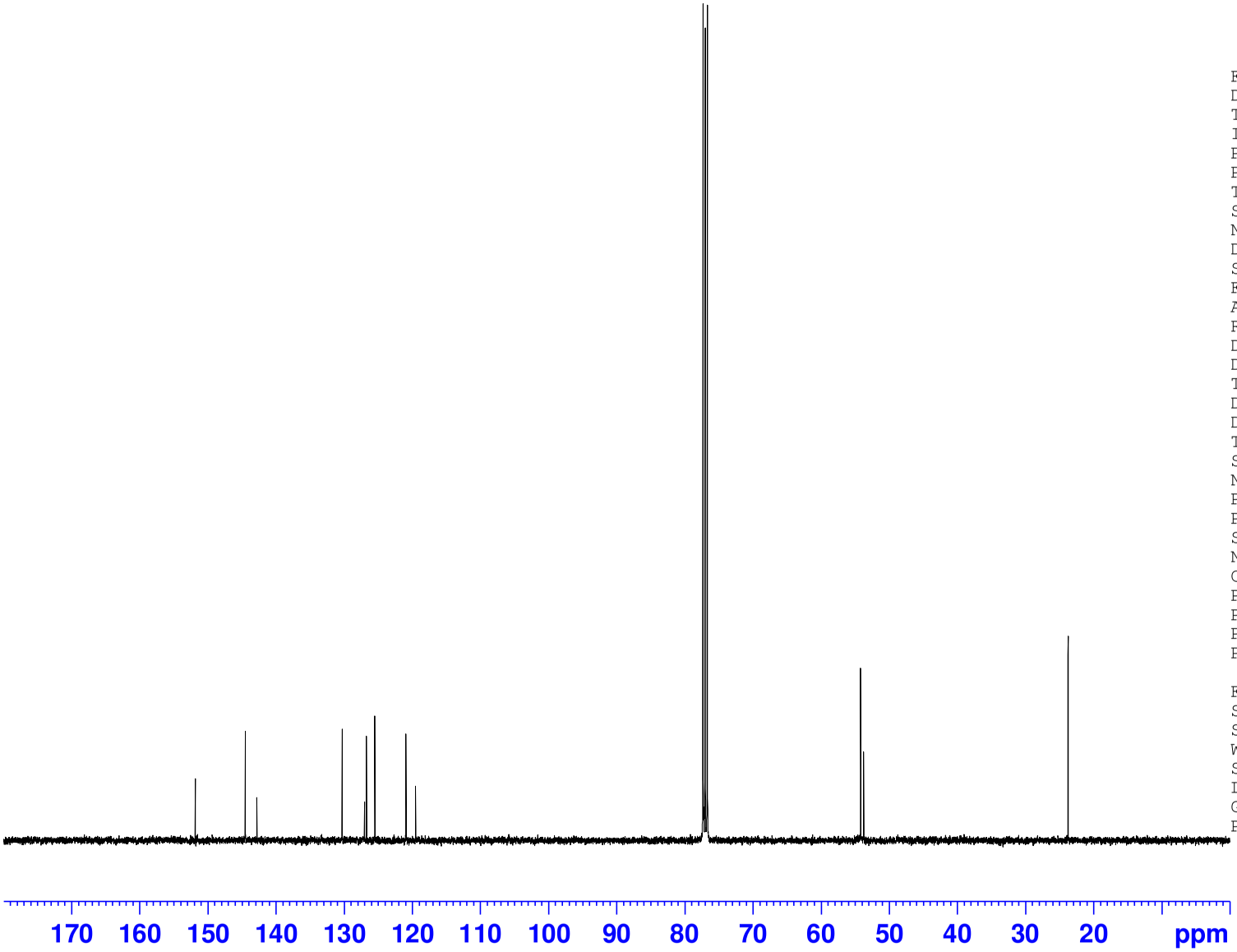
Faculty Group Callam

151.84
144.51
142.83
130.30
127.02
126.74
125.51
120.93
119.52
54.20
53.76
23.74

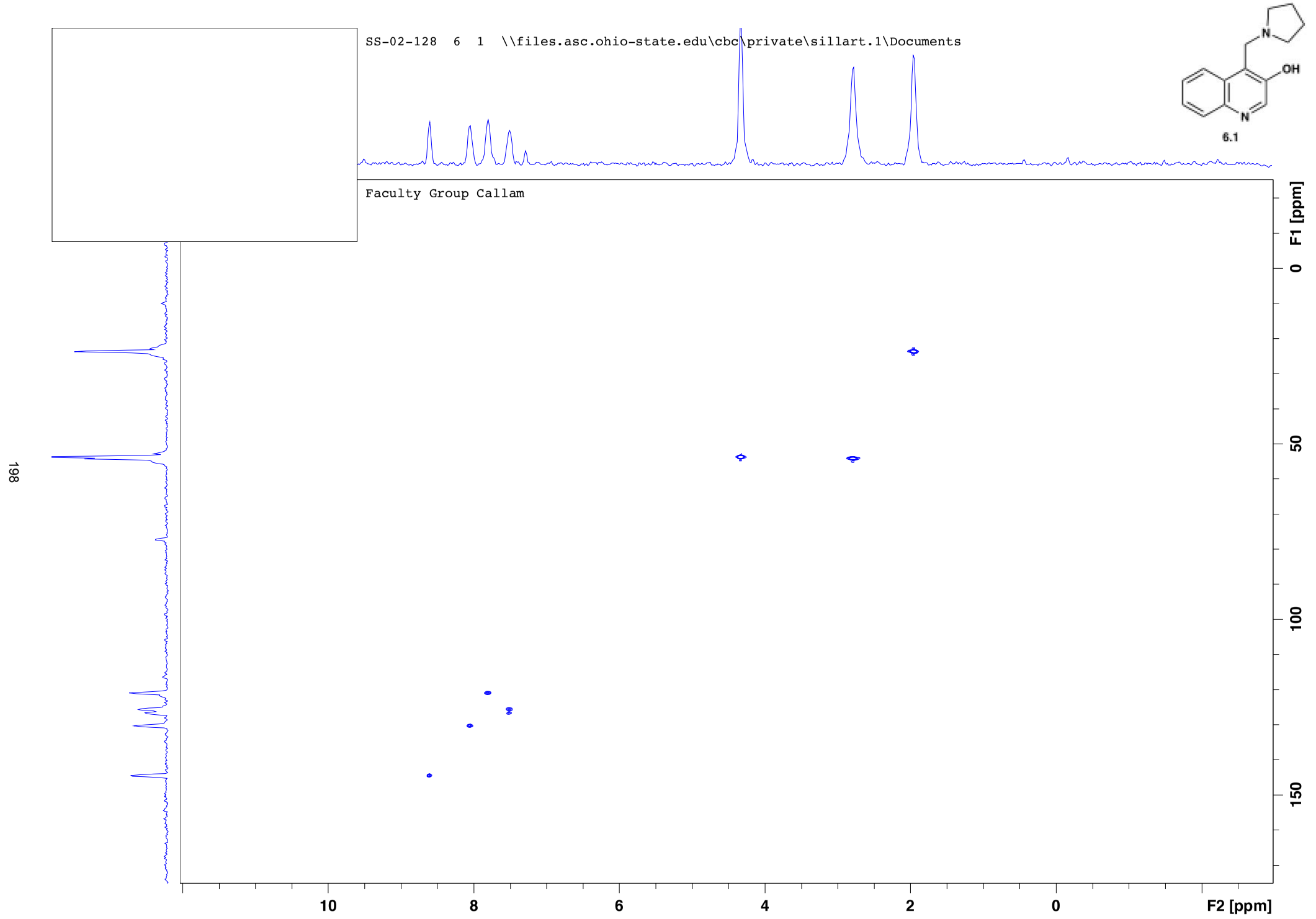


6.1

197



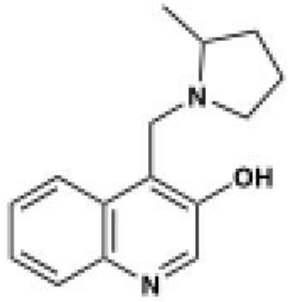

```
F2 - Acquisition Parameters
Date_           20180114
Time              17.59 h
INSTRUM             spect
PROBHD   Z108618_0433 (
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE               301.6 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1
SFO1         100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1         53.29999924 W
SFO2         400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.60000038 W
PLW12         0.40555999 W
PLW13         0.20399000 W

F2 - Processing parameters
SI                32768
SF           100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```


SS-02-128 6 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.1
F1 [ppm]
0
50
100
150
10
8
6
4
2
0
F2 [ppm]

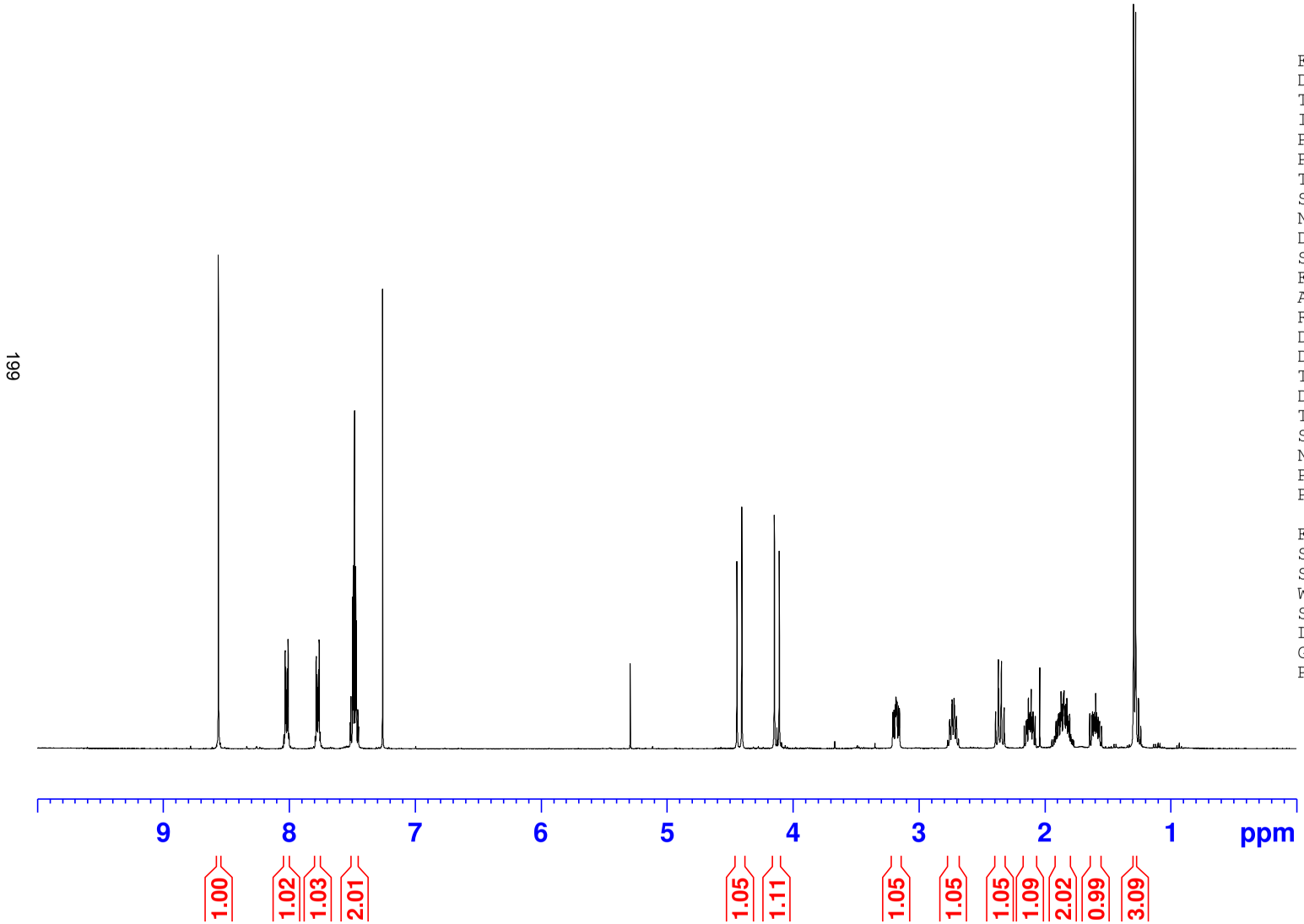
Faculty Group Callam

8.563 8.028 8.026 8.020 7.783 7.777 7.768 7.483

4.445 4.406 4.148 4.109 3.207 3.199 3.188 3.182 3.179 3.174 3.163 3.154 2.771 2.756 2.737 2.720 2.701 2.686 2.390 2.369 2.344 2.323 2.118 1.865 1.856 1.596



6.2



F2 - Acquisition Parameters

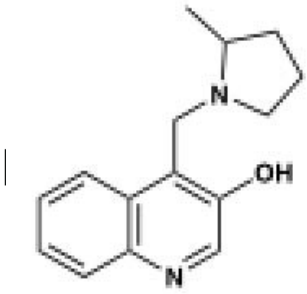
Date_	20180129	
Time	19.28	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

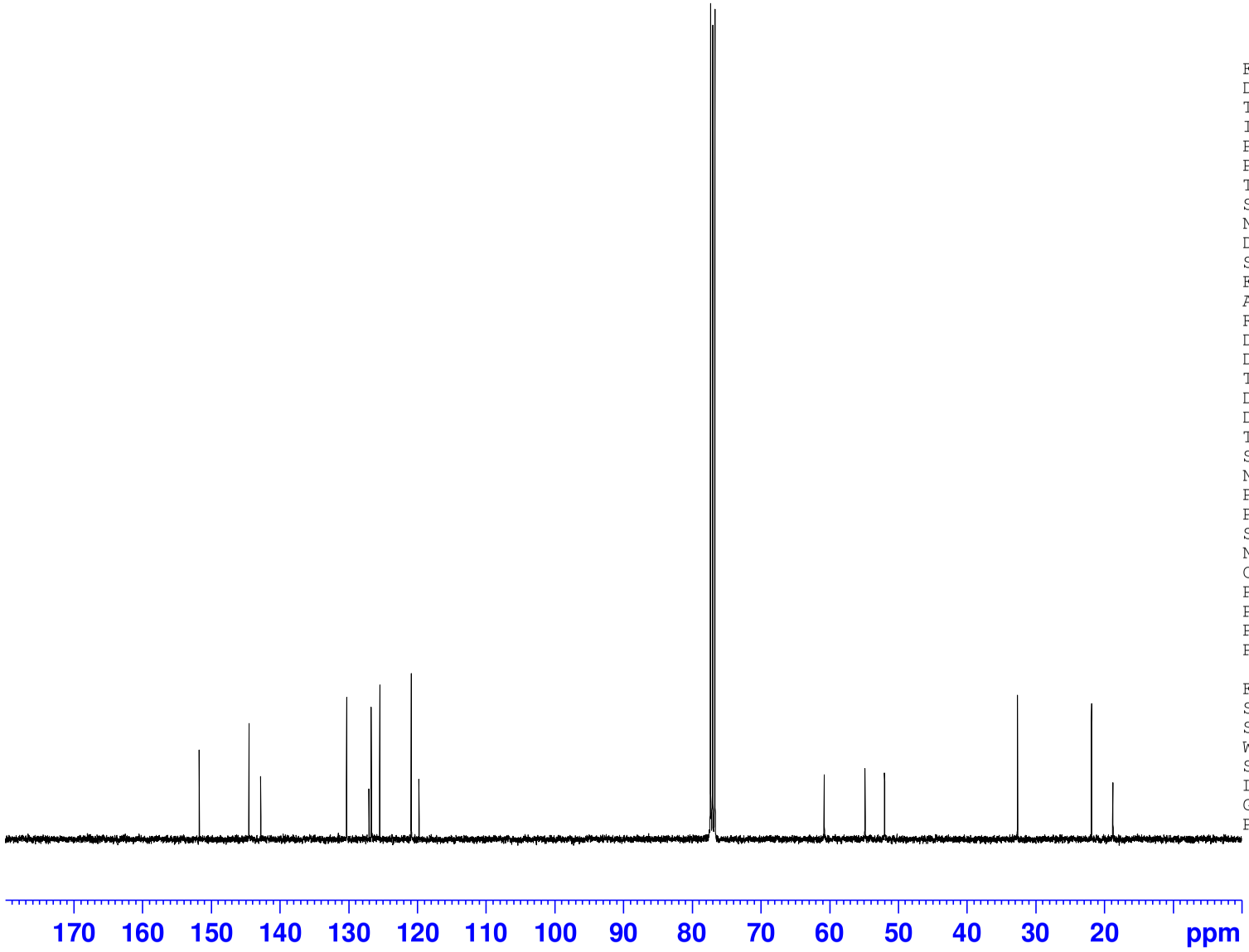
SI	65536	
SF	400.1700098	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

151.77
144.53
142.81
130.32
127.06
126.73
125.49
120.91
119.77
60.78
54.85
52.00
32.65
21.87
18.76



6.2

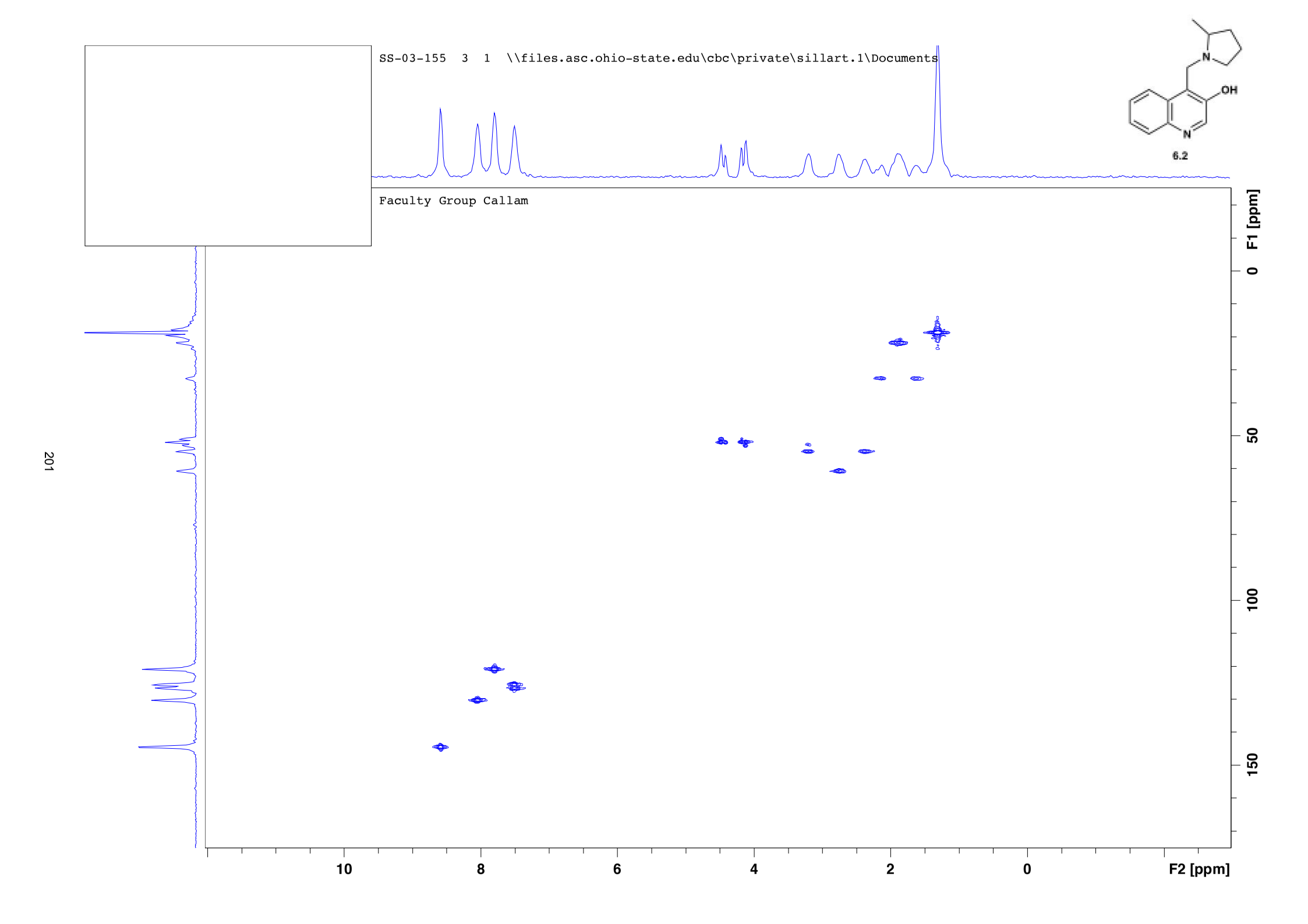


F2 - Acquisition Parameters

Date_	20180130	
Time	2.38	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

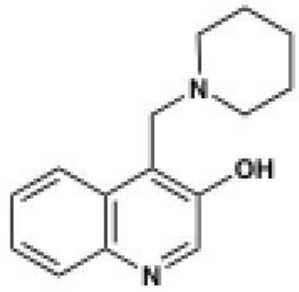
F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

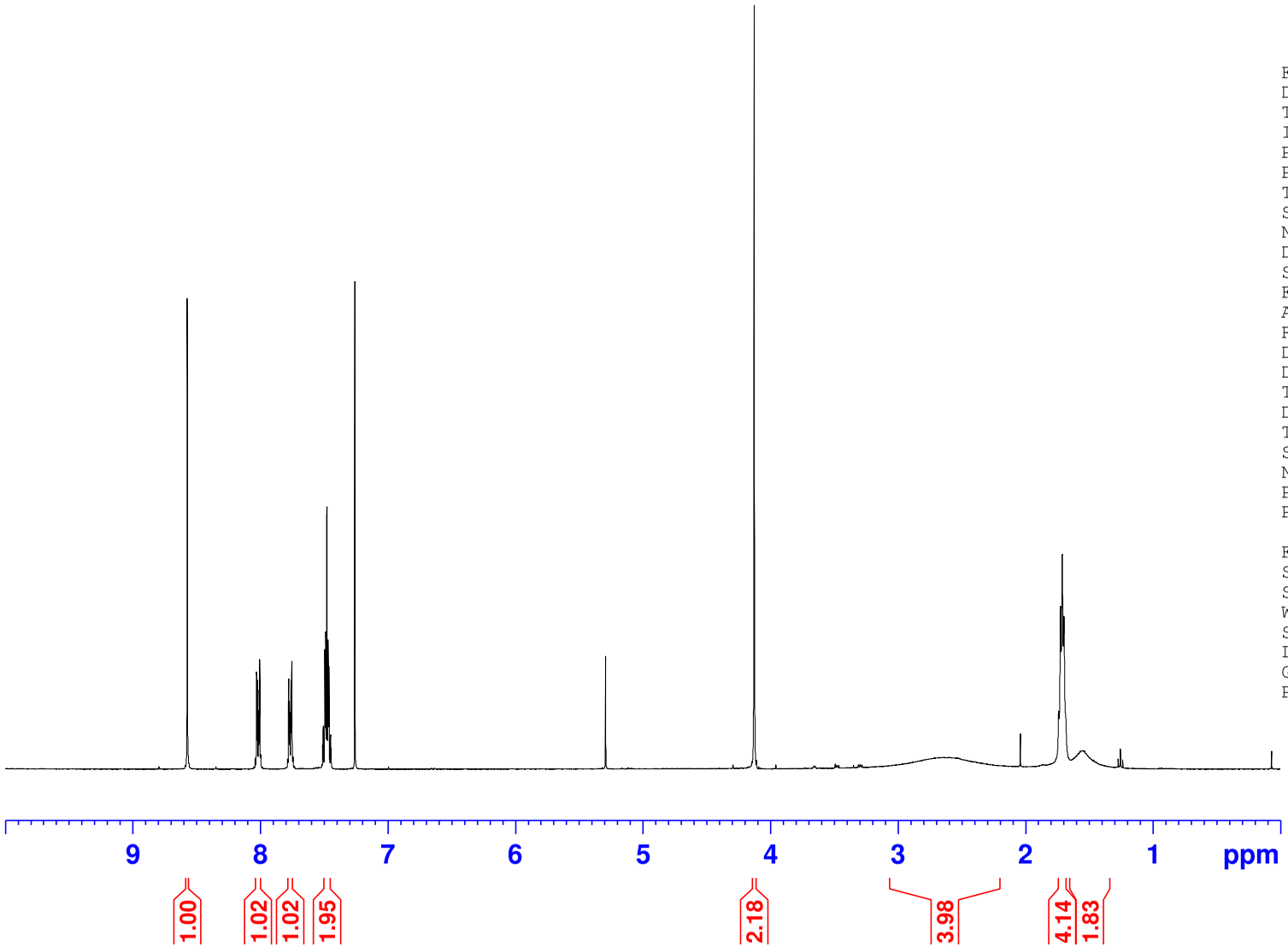

SS-03-155 3 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.2
F1 [ppm]
0
50
100
150
F2 [ppm]
10
8
6
4
2
0
201

Faculty Group Callam

8.574
8.030
8.025
8.010
8.006
7.779
7.760
7.479
4.128
2.618
1.726
1.712
1.699
1.574



6.3



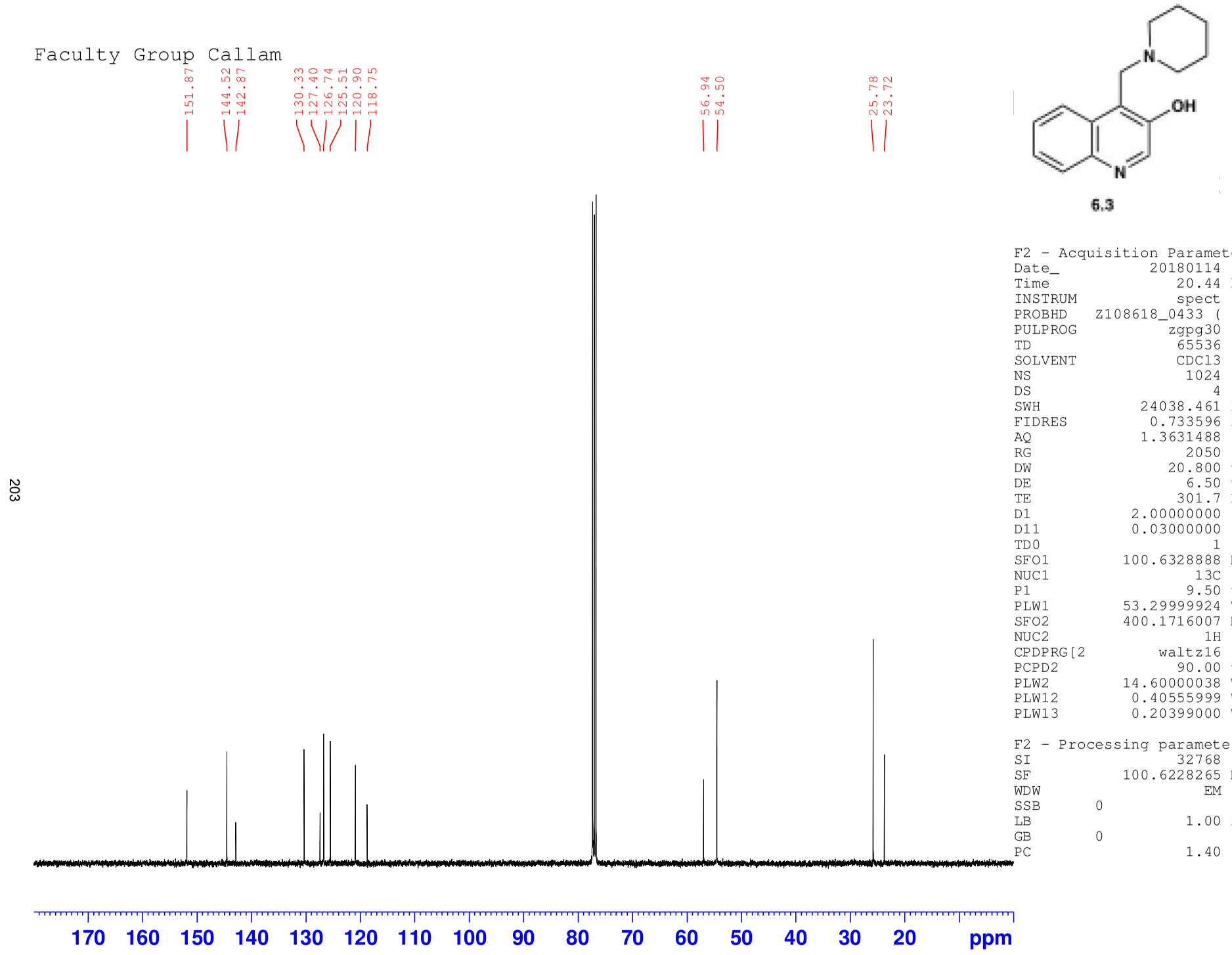
F2 - Acquisition Parameters

Date_	20180114	
Time	19.42	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	219.29	
DW	60.800	usec
DE	6.50	usec
TE	301.0	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700097	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam



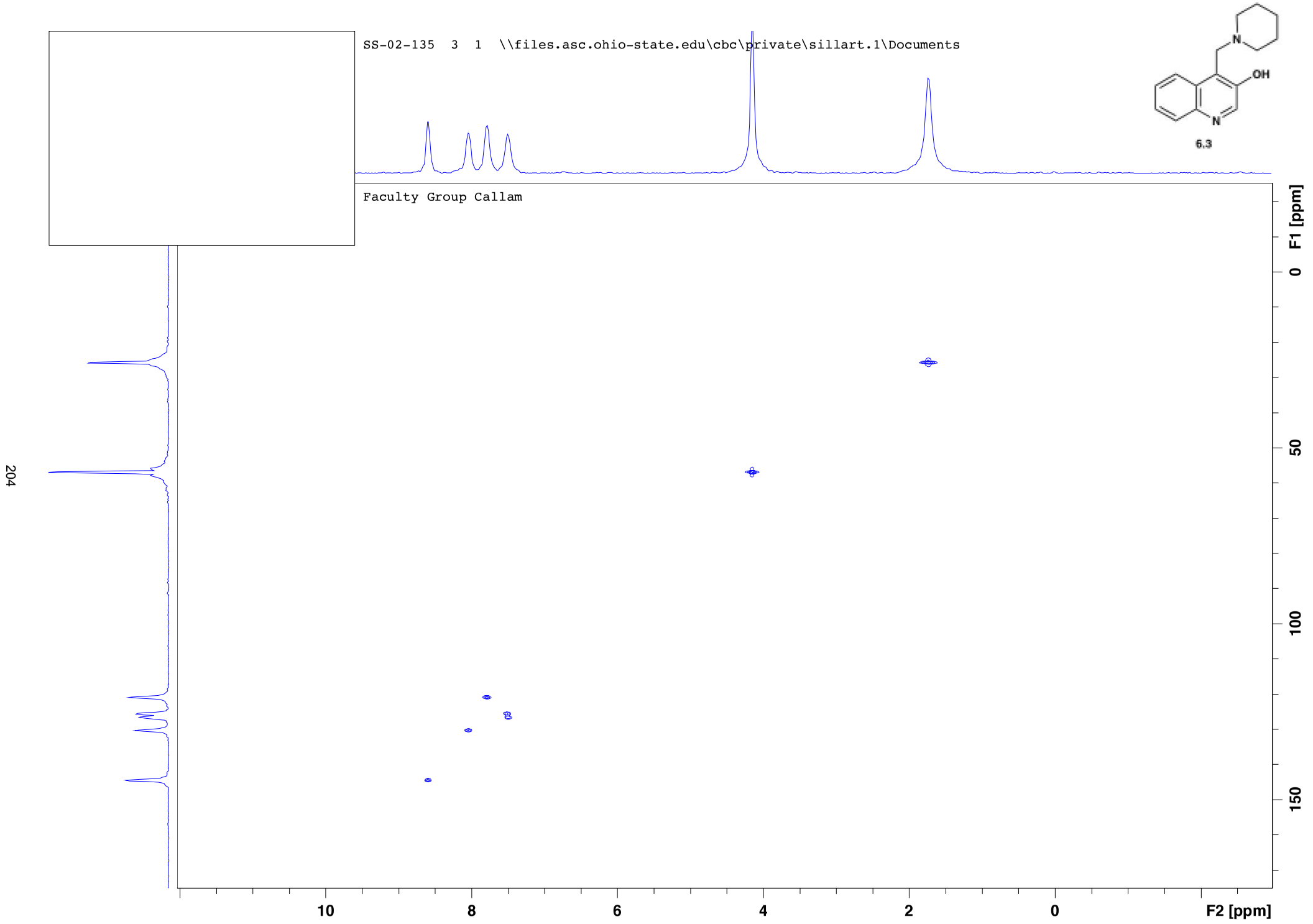
6.3

F2 - Acquisition Parameters

Date_	20180114	
Time	20.44	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	301.7	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	


SS-02-135 3 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.3
F1 [ppm]
0
50
100
150
F2 [ppm]
10
8
6
4
2
0
204

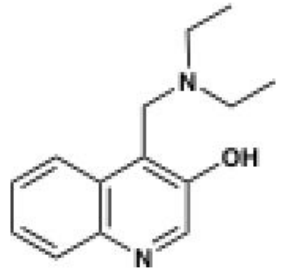
Faculty Group Callam

8.558
8.019
8.013
8.007
7.763
7.758
7.752
7.749
7.491
7.485
7.476
7.466
7.460

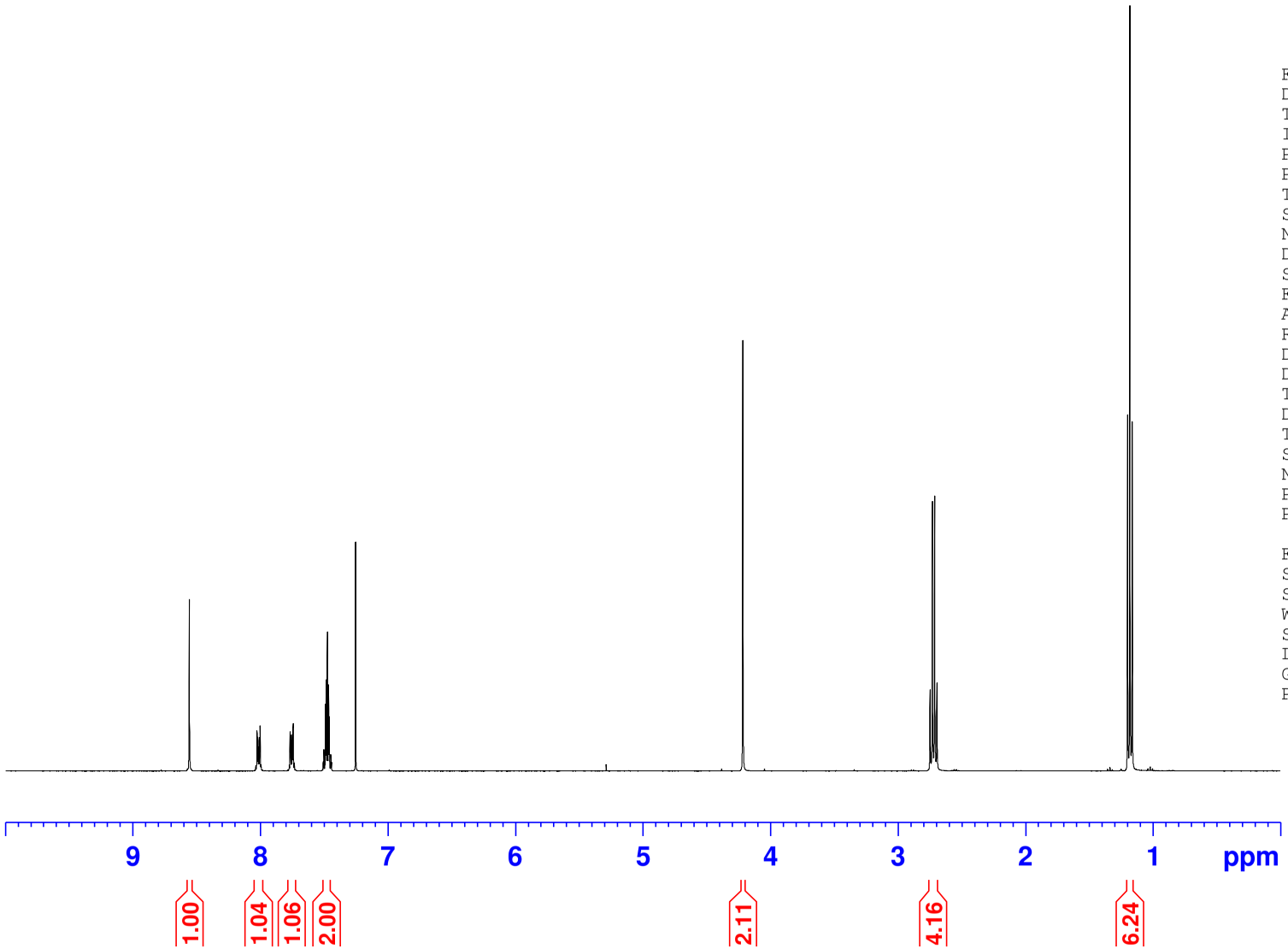
4.217

2.749
2.731
2.713
2.695

1.200
1.182
1.164



6.4



205

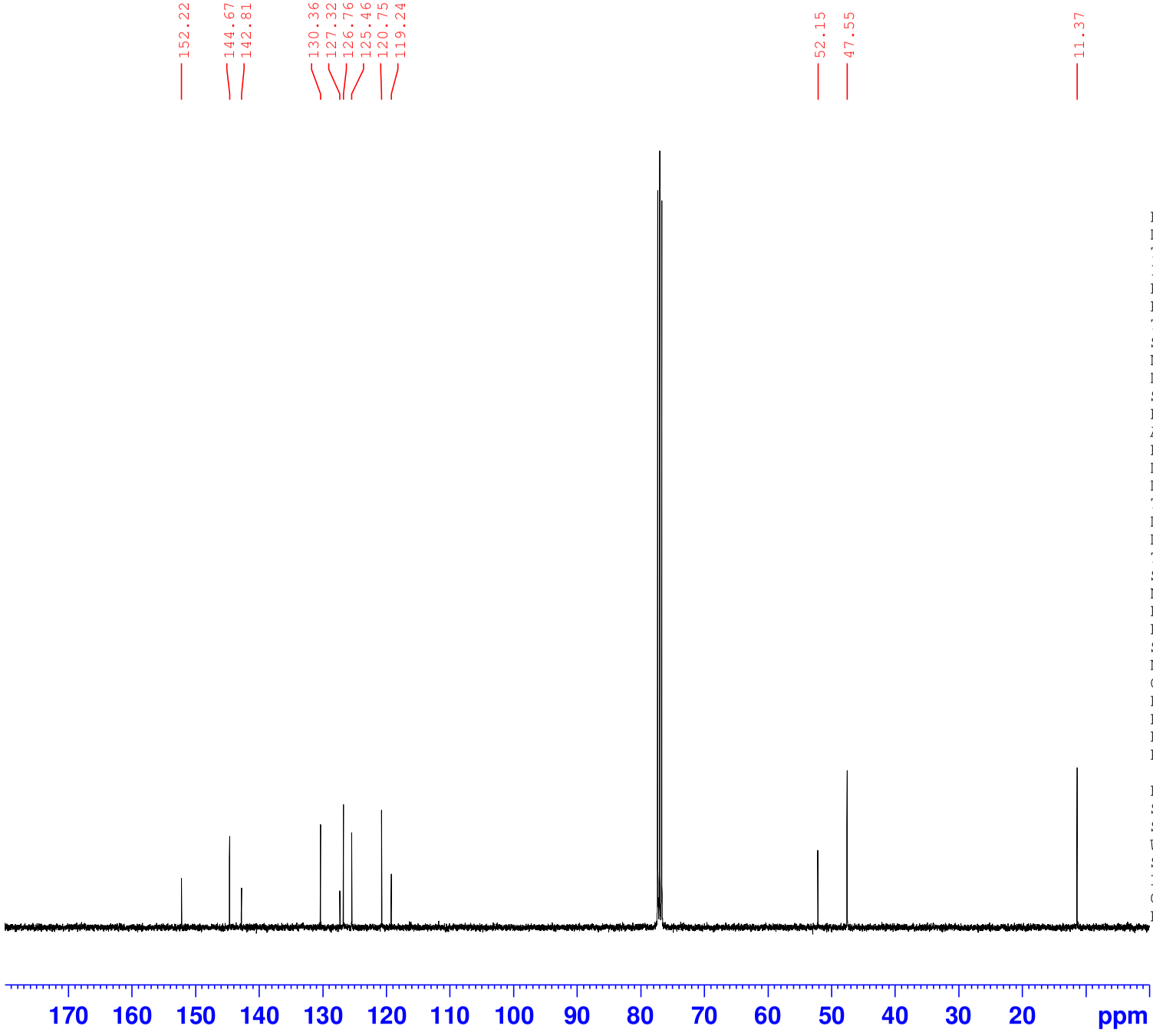
F2 - Acquisition Parameters

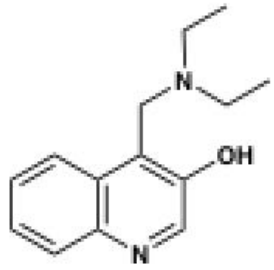
Date_	20180114	
Time	20.49	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	301.2	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700124	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





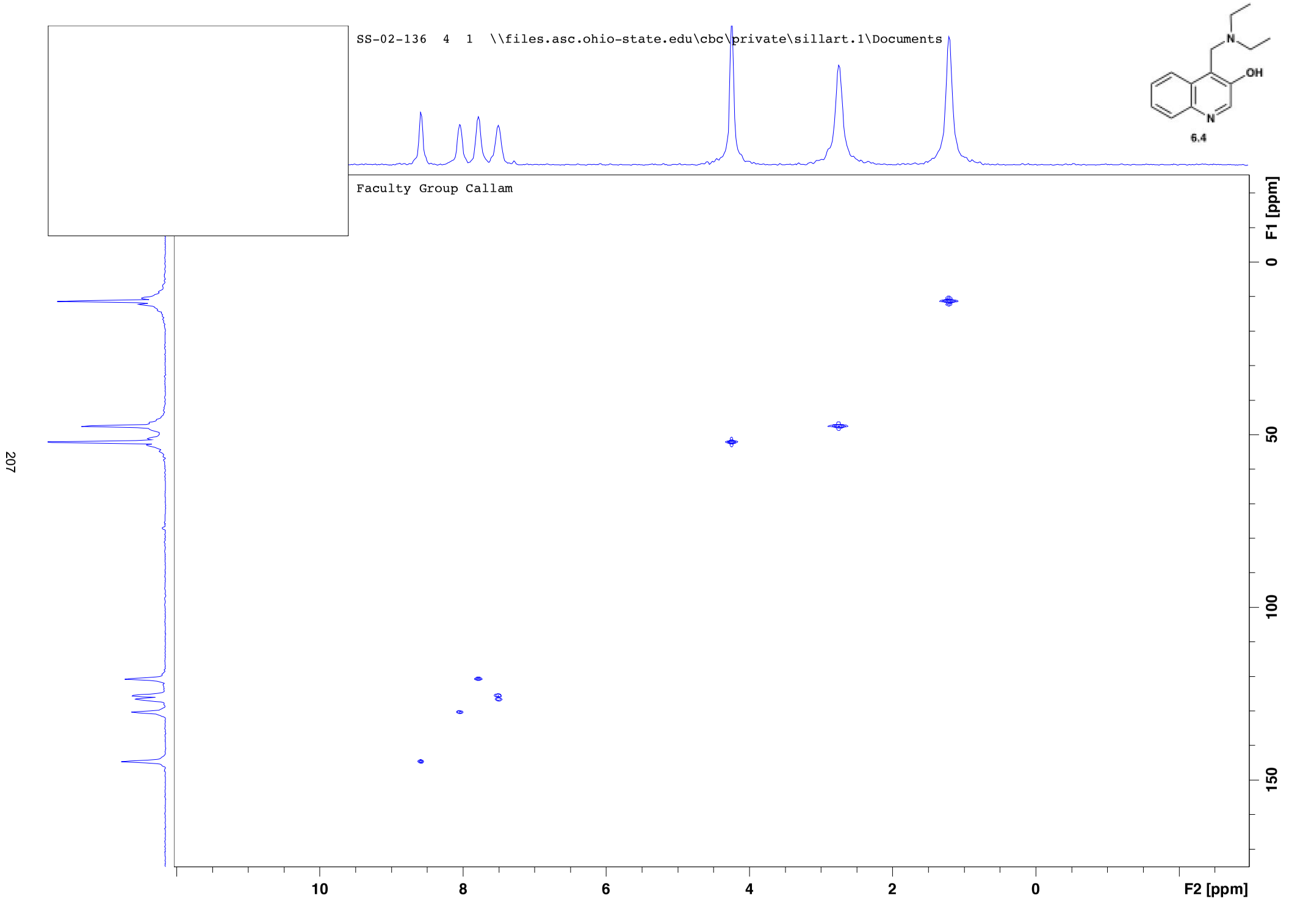
6.4

F2 - Acquisition Parameters

Date_	20180114	
Time	21.50	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	301.7	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

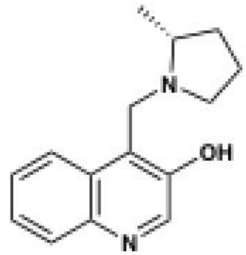
F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

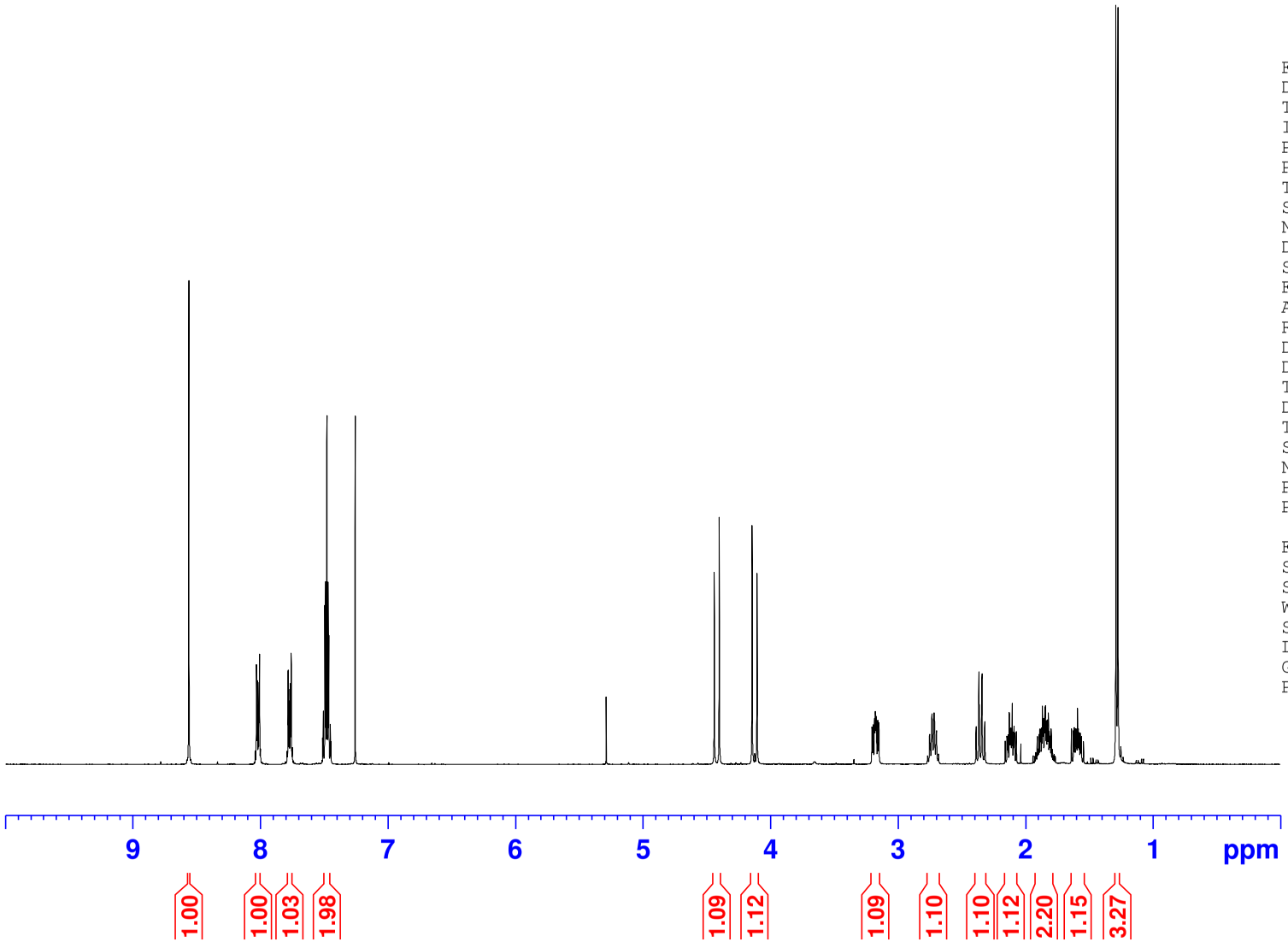

SS-02-136 4 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.4
F1 [ppm]
0
50
100
150
F2 [ppm]
10
8
6
4
2
0
207

Faculty Group Callam

8.562 8.031 8.026 8.023 8.017 8.011 7.779 7.774 7.767 7.765 7.479 4.441 4.402 4.144 4.106 3.204 3.195 3.184 3.179 3.176 3.170 3.160 3.151 2.752 2.733 2.717 2.698 2.387 2.366 2.341 2.320 2.127 2.123 2.118 2.114 2.104 1.868 1.861 1.853 1.847 1.843 1.592 1.291 1.276



6.5



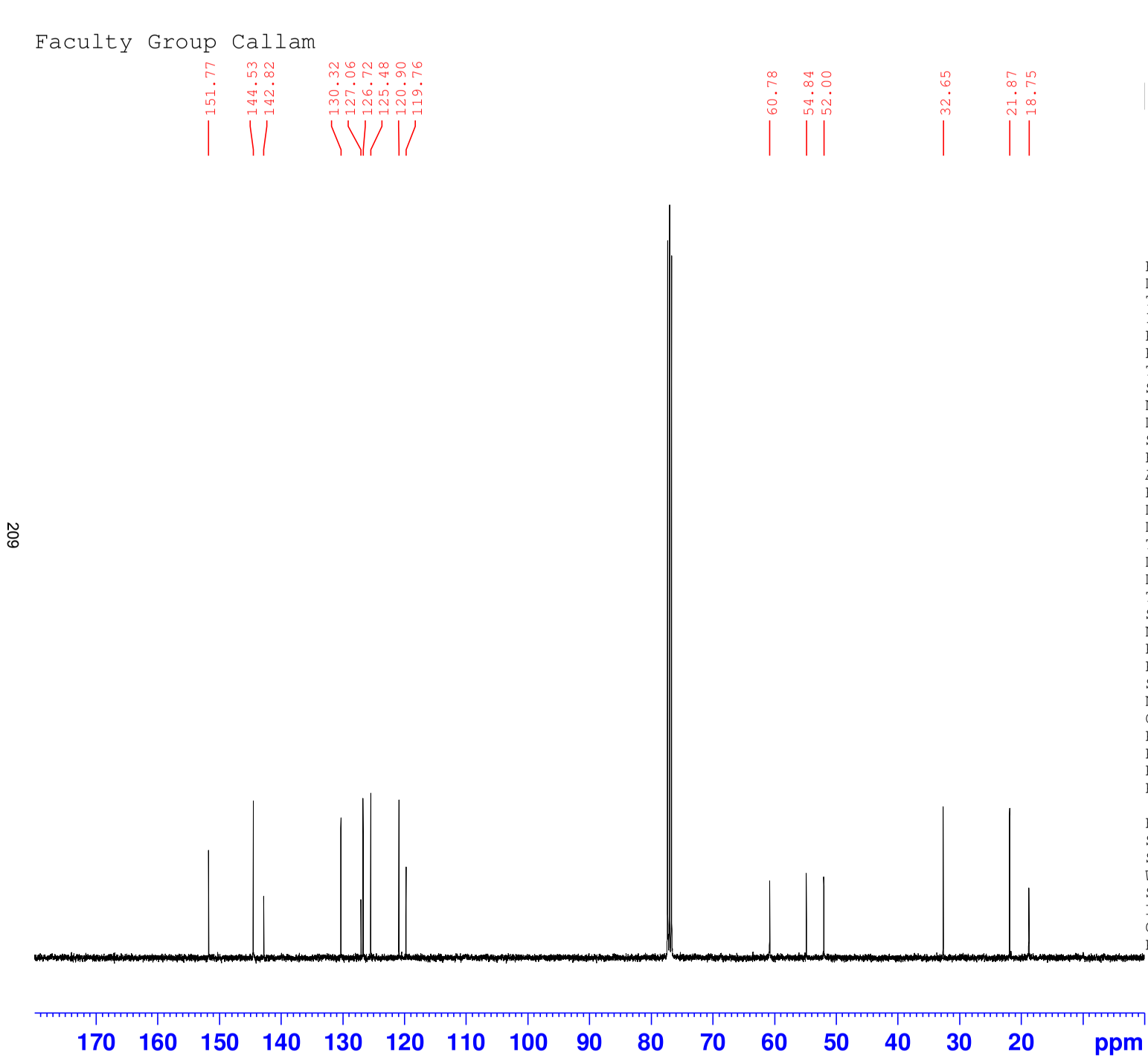
F2 - Acquisition Parameters

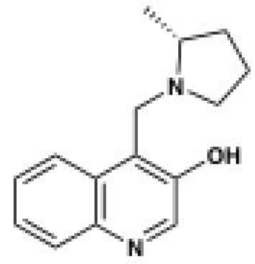
Date_	20180114	
Time	18.13	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	188.13	
DW	60.800	usec
DE	6.50	usec
TE	301.0	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700111	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam





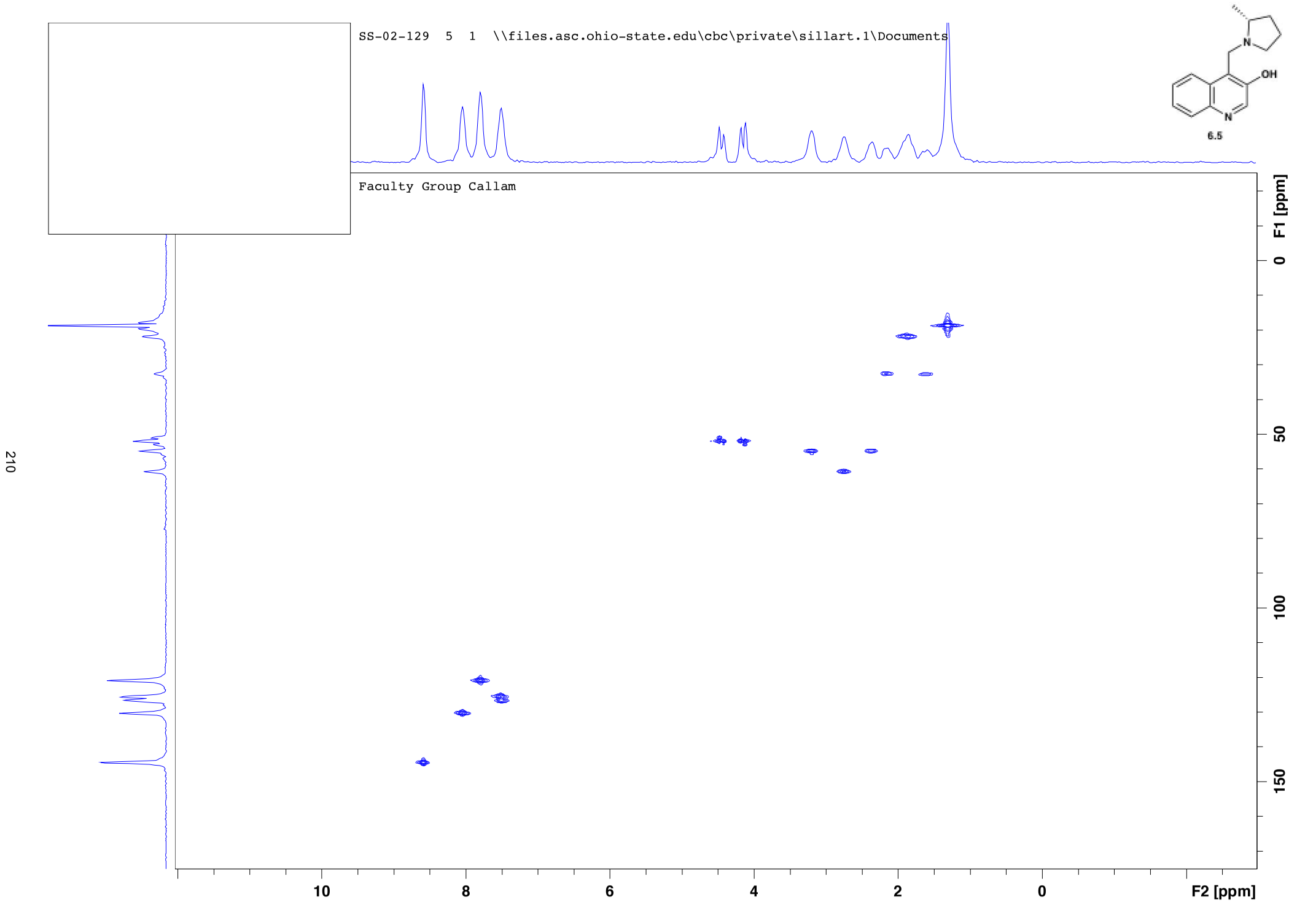
6.5

F2 - Acquisition Parameters

Date_	20180114	
Time	19.13	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	301.7	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

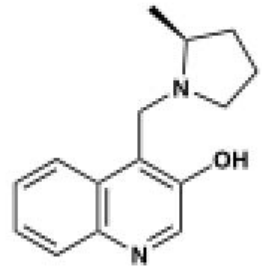
SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	


SS-02-129 5 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.5
210
F1 [ppm]
0
50
100
150
10
8
6
4
2
0
F2 [ppm]

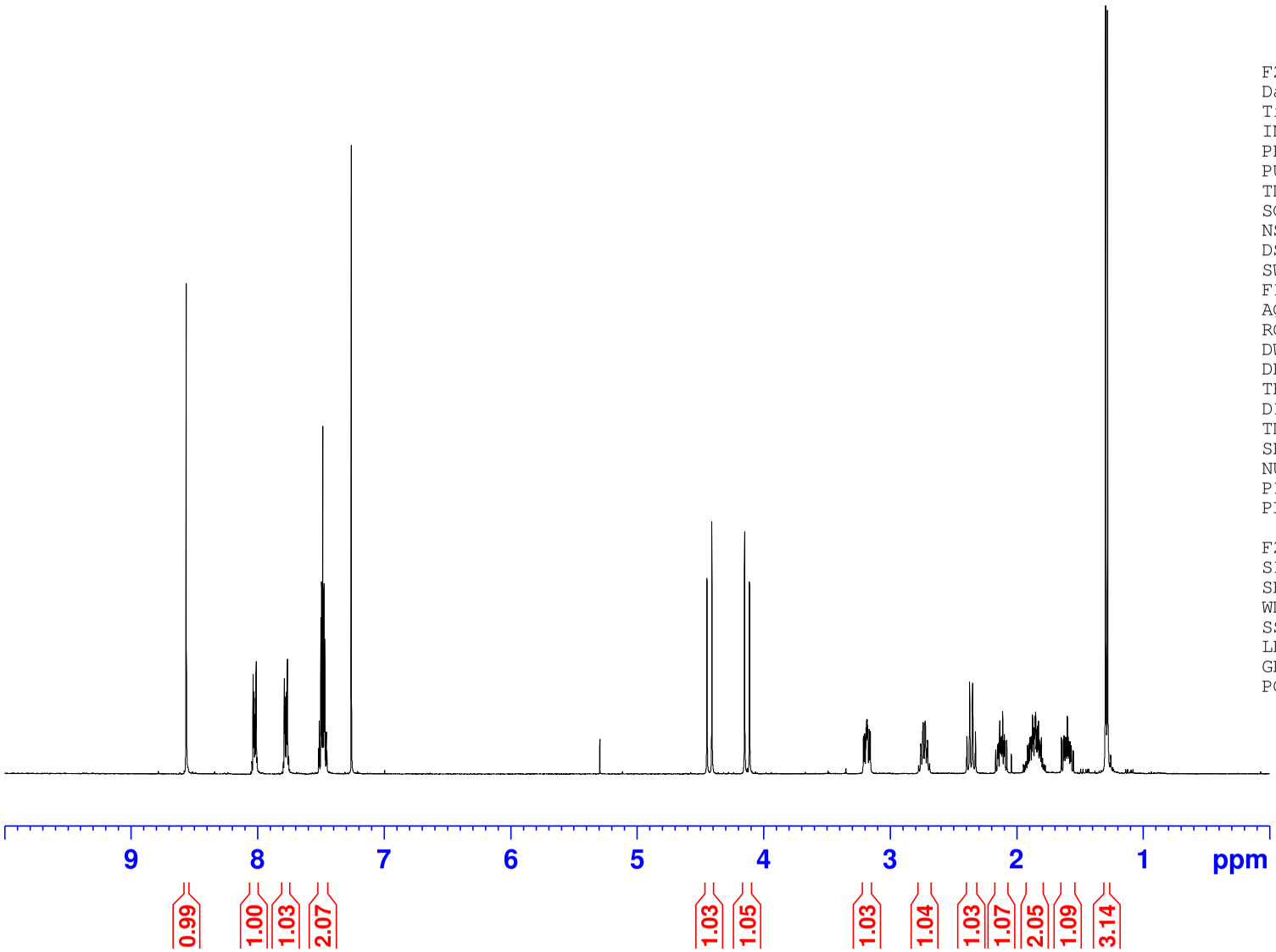
Faculty Group Callam

8.565 8.030 8.028 8.016 7.785 7.771 7.765 7.485 7.260

4.448 4.409 4.150 4.112 3.210 3.201 3.191 3.185 3.182 3.177 3.166 3.157 2.774 2.758 2.739 2.723 2.704 2.688 2.393 2.372 2.347 2.326 2.133 2.110 1.874 1.853



6.6



211

F2 - Acquisition Parameters

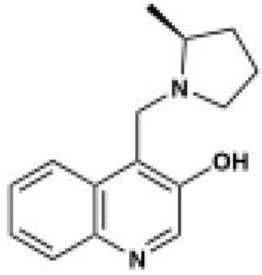
Date_	20180129	
Time	19.22	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	244.73	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

SI	65536	
SF	400.1700096	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

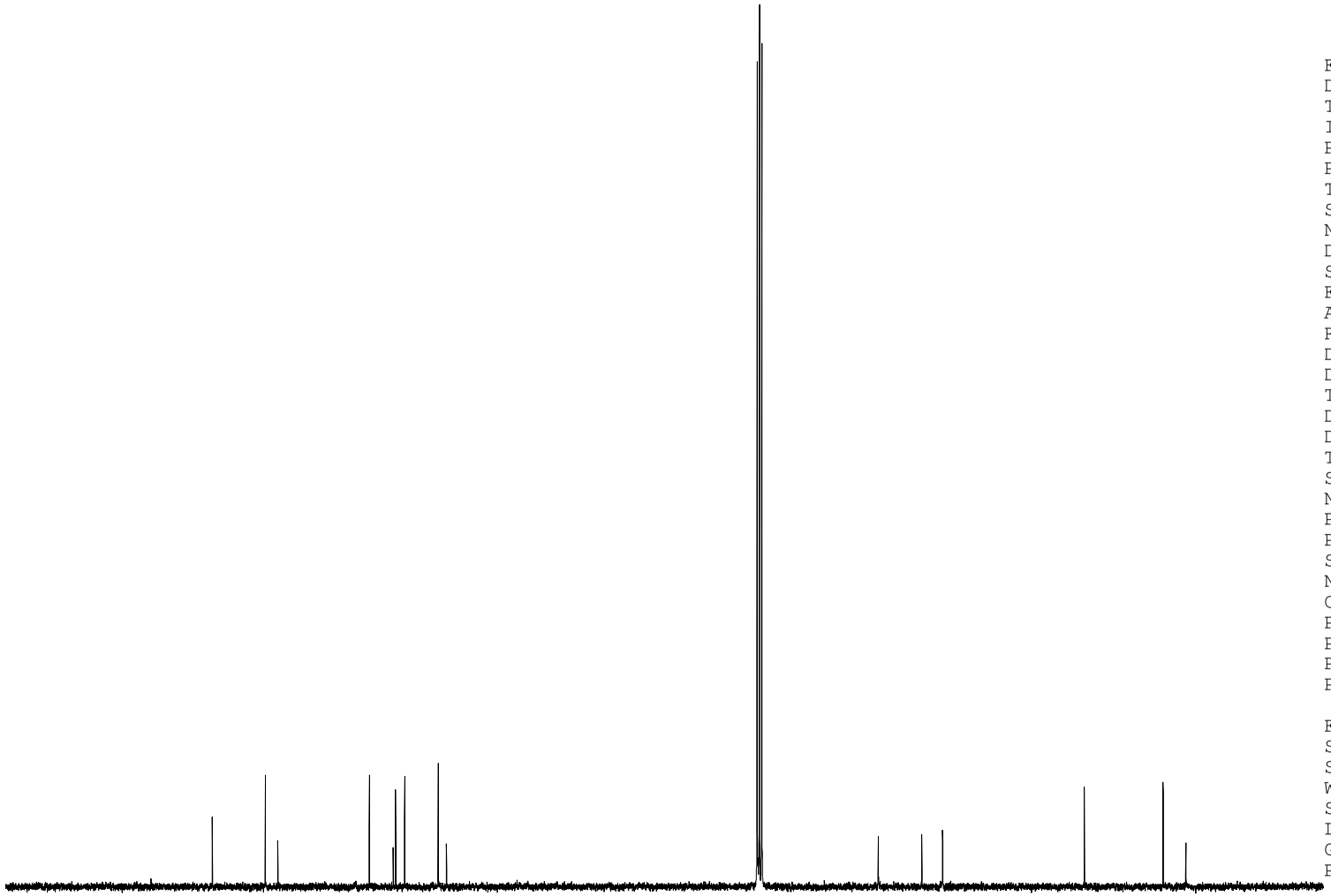
Faculty Group Callam

151.77
144.53
142.81
130.32
127.06
126.73
125.49
120.91
119.77
60.78
54.85
52.00
32.65
21.88
18.76



6.6

212



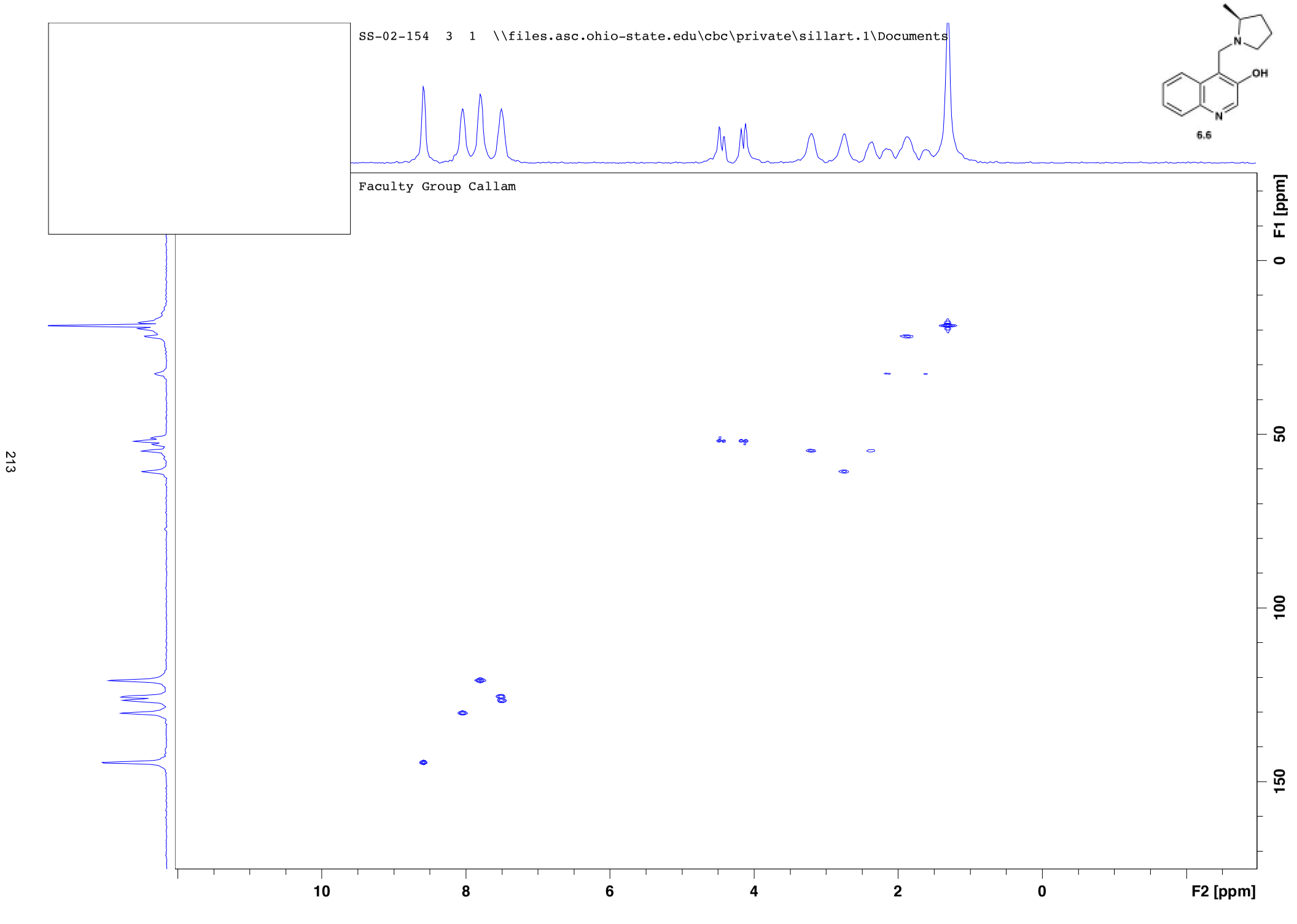
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

F2 - Acquisition Parameters

Date_	20180130	
Time	1.18	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	1024	
DS	4	
SWH	24038.461	Hz
FIDRES	0.733596	Hz
AQ	1.3631488	sec
RG	2050	
DW	20.800	usec
DE	6.50	usec
TE	300.5	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
SFO1	100.6328888	MHz
NUC1	13C	
P1	9.50	usec
PLW1	53.29999924	W
SFO2	400.1716007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	90.00	usec
PLW2	14.60000038	W
PLW12	0.40555999	W
PLW13	0.20399000	W

F2 - Processing parameters

SI	32768	
SF	100.6228265	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

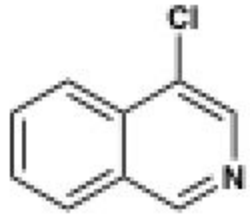

SS-02-154 3 1 \\files.asc.ohio-state.edu\cbc\private\sillart.1\Documents
Faculty Group Callam
OH
N
N
6.6
F1 [ppm]
0
50
100
150
F2 [ppm]
10
8
6
4
2
0

APPENDIX F

SELECT ^{1}HNMR AND ^{13}CNMR DATA FROM CHAPTER 7

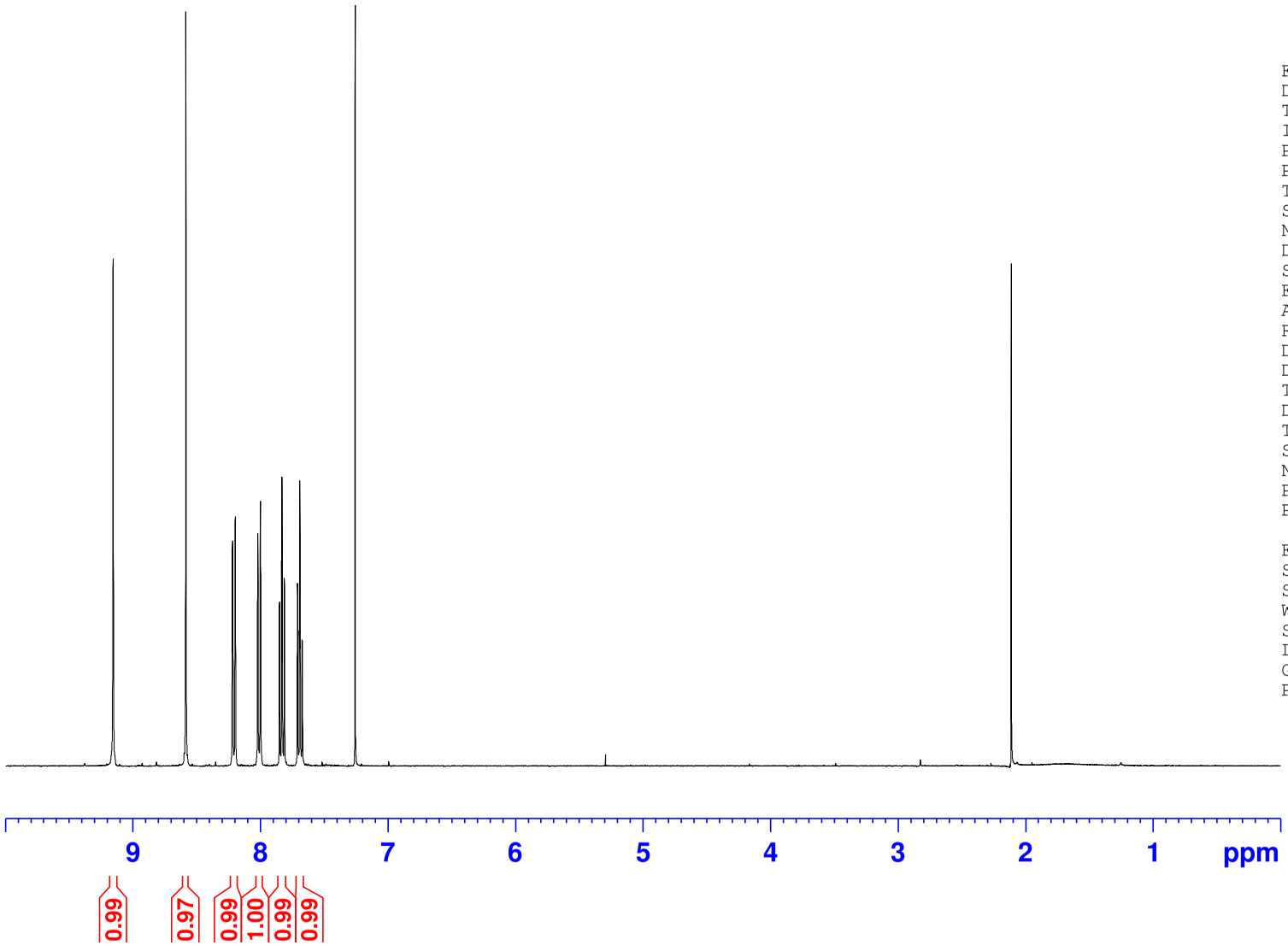
Faculty Group Callam

9.156
8.585
8.197
8.000
7.831
7.691



7.3

215



F2 - Acquisition Parameters

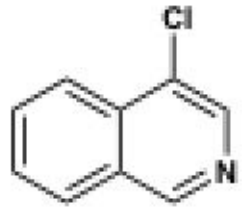
Date_	20180123	
Time	9.52	h
INSTRUM	spect	
PROBHD	Z108618_0433 (	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	3.9845889	sec
RG	273.81	
DW	60.800	usec
DE	6.50	usec
TE	300.5	K
D1	1.00000000	sec
TD0	1	
SFO1	400.1724712	MHz
NUC1	1H	
P1	15.00	usec
PLW1	13.19999981	W

F2 - Processing parameters

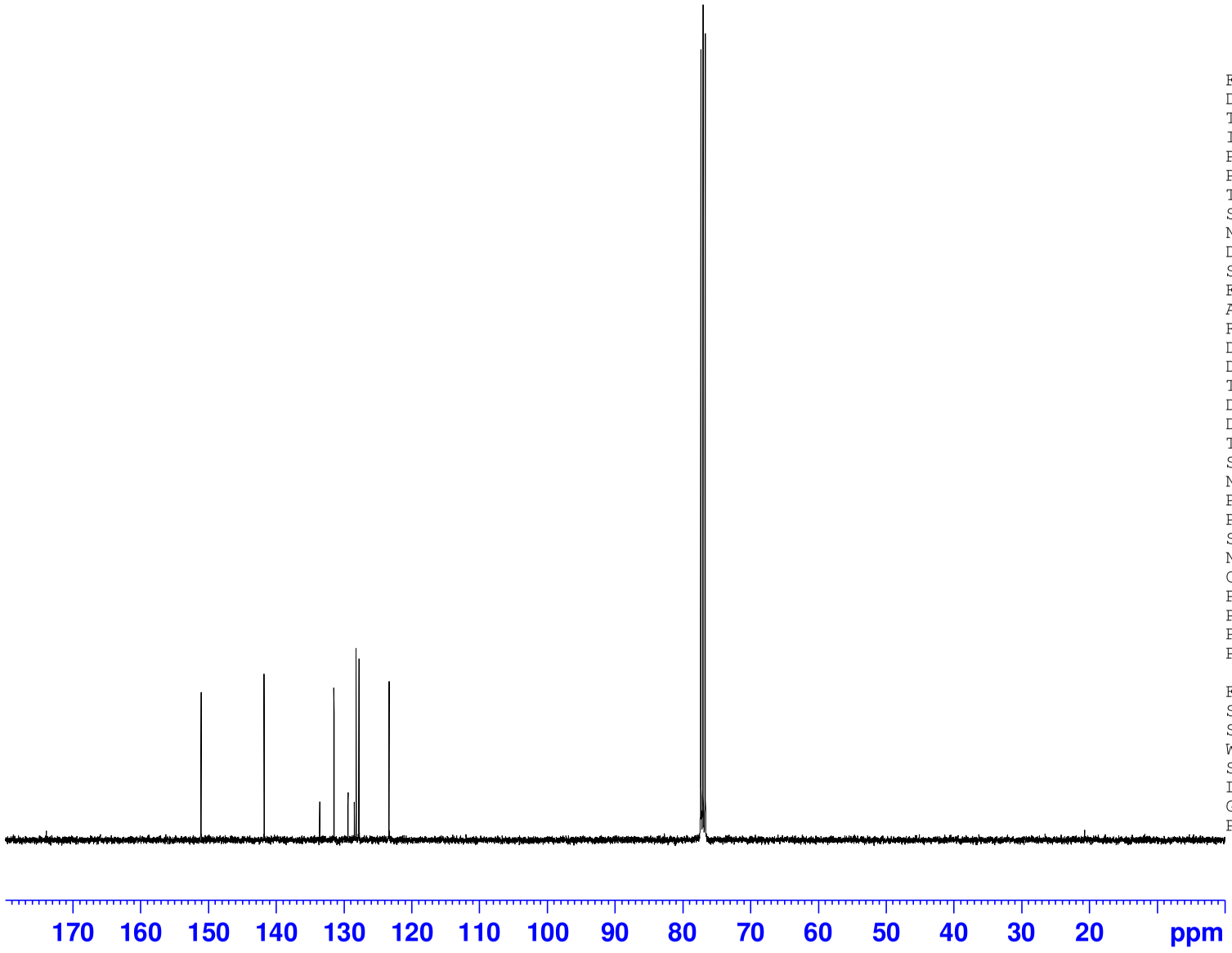
SI	65536	
SF	400.1700110	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Faculty Group Callam

151.09
141.79
133.58
131.48
129.42
128.49
128.21
127.78
123.34



7.3



216

```
F2 - Acquisition Parameters
Date_           20180126
Time              23.46 h
INSTRUM             spect
PROBHD   Z108618_0433 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                300.5 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6328888 MHz
NUC1                13C
P1                 9.50 usec
PLW1        53.29999924 W
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        14.60000038 W
PLW12        0.40555999 W
PLW13        0.20399000 W

F2 - Processing parameters
SI                32768
SF          100.6228265 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

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